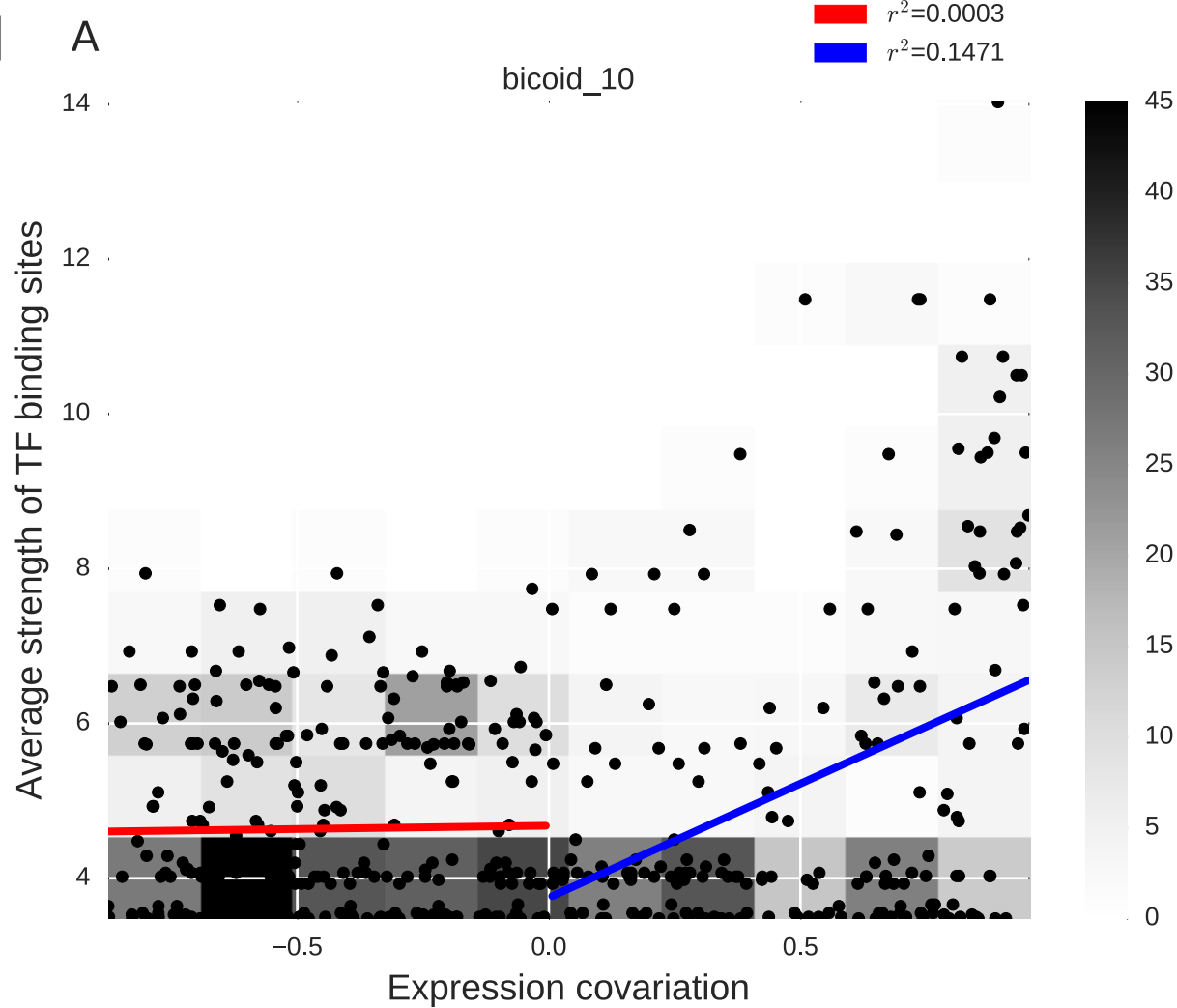
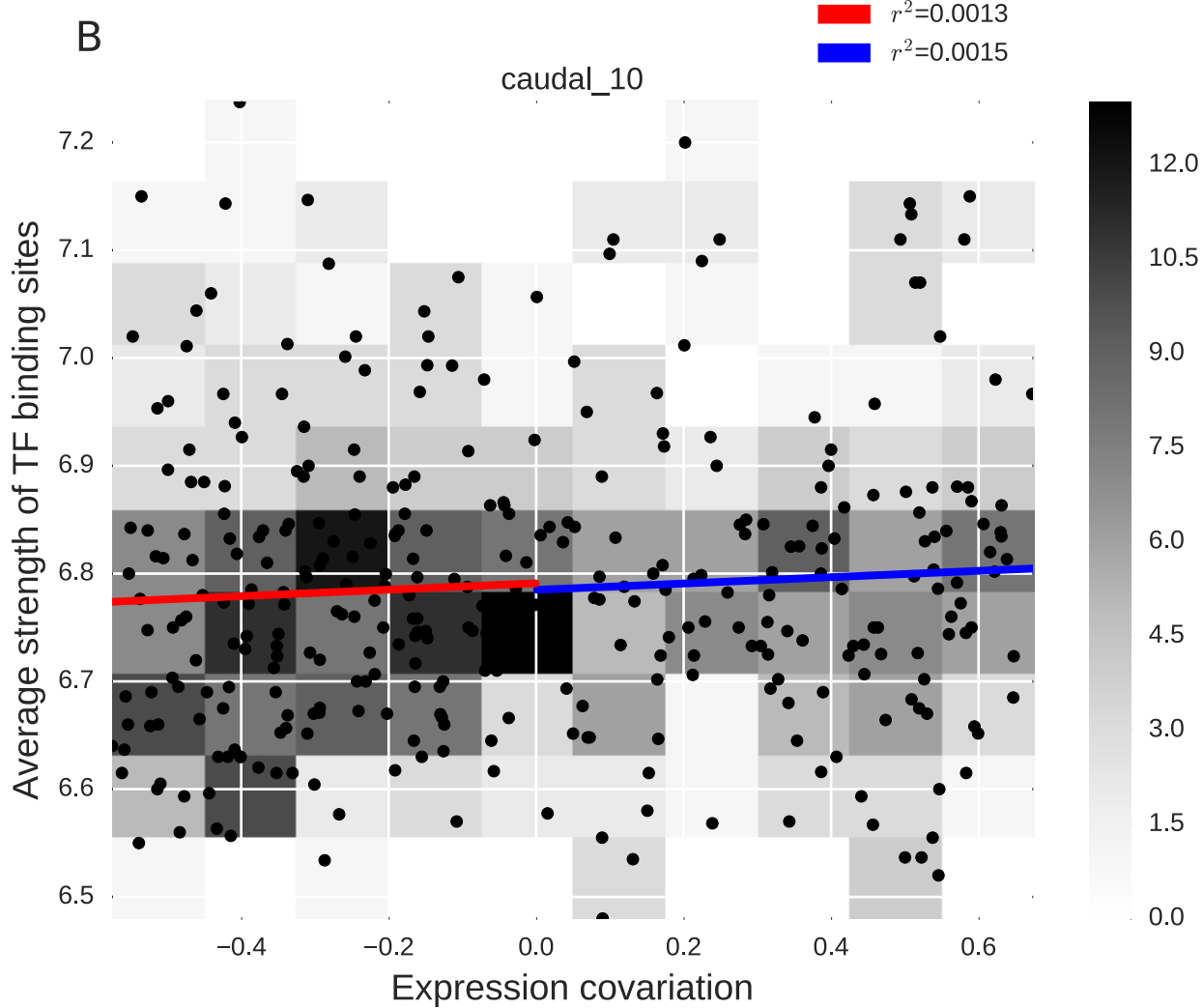


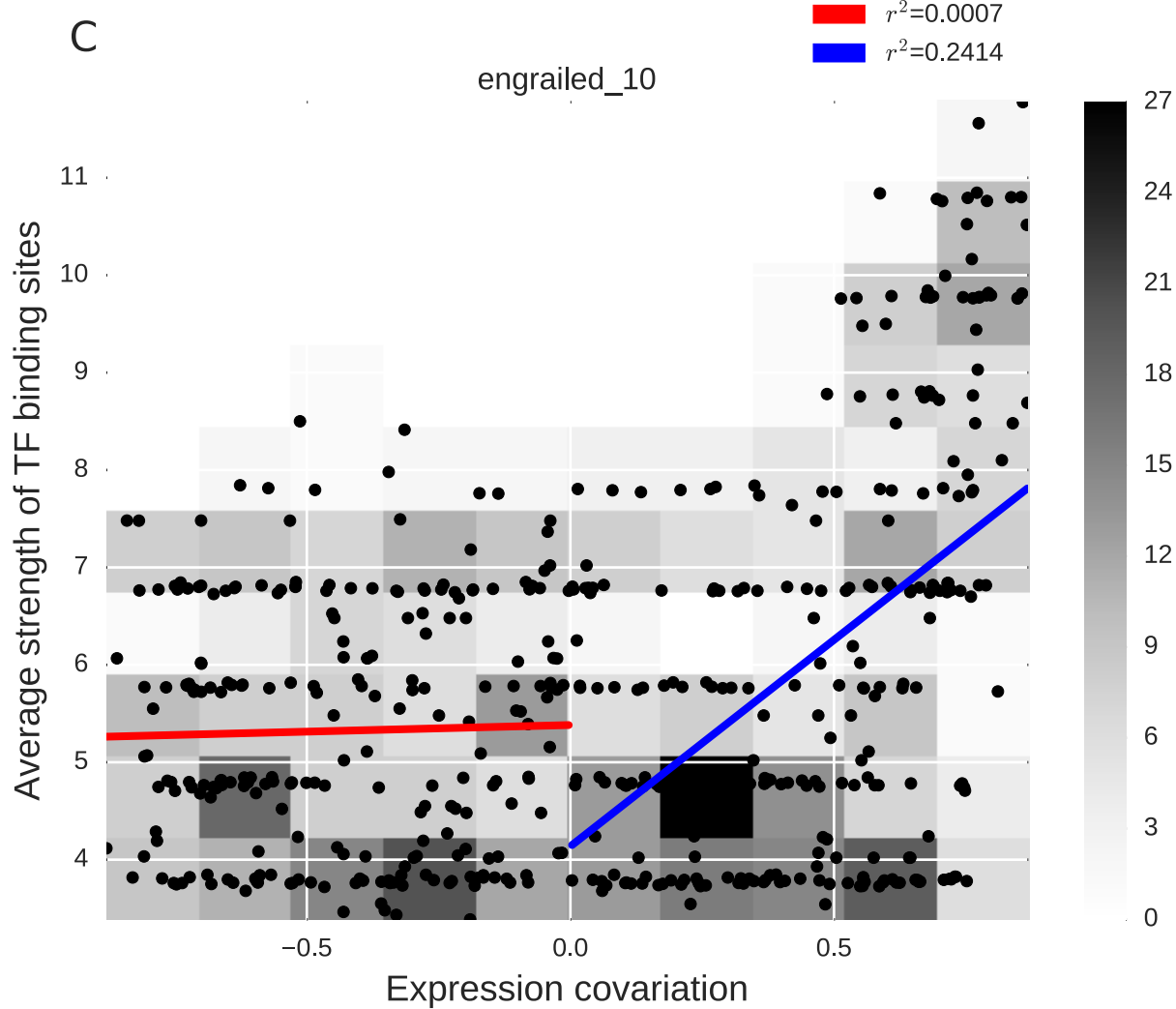
**Figure S1:** The relationship between regulatory strength and average TF binding site strength for target genes using the top 10% of binding scores for each TF. Here we plot the relationship between regulatory strength and average TF binding strength for all our TFs of interest. Each black dot represents a target for the TF in the panel with at least three binding motifs. The x-axis shows the correlations between the expression level of each TF and its target genes across all our samples. The y-axis shows the average TF binding site strength for binding sites located in the assumed region of regulatory DNA for each target gene. The blue and red lines show the linear regression for the positively and negatively correlated target genes, respectively, and  $r^2$  values are displayed for these best fit lines.

**S1****A**

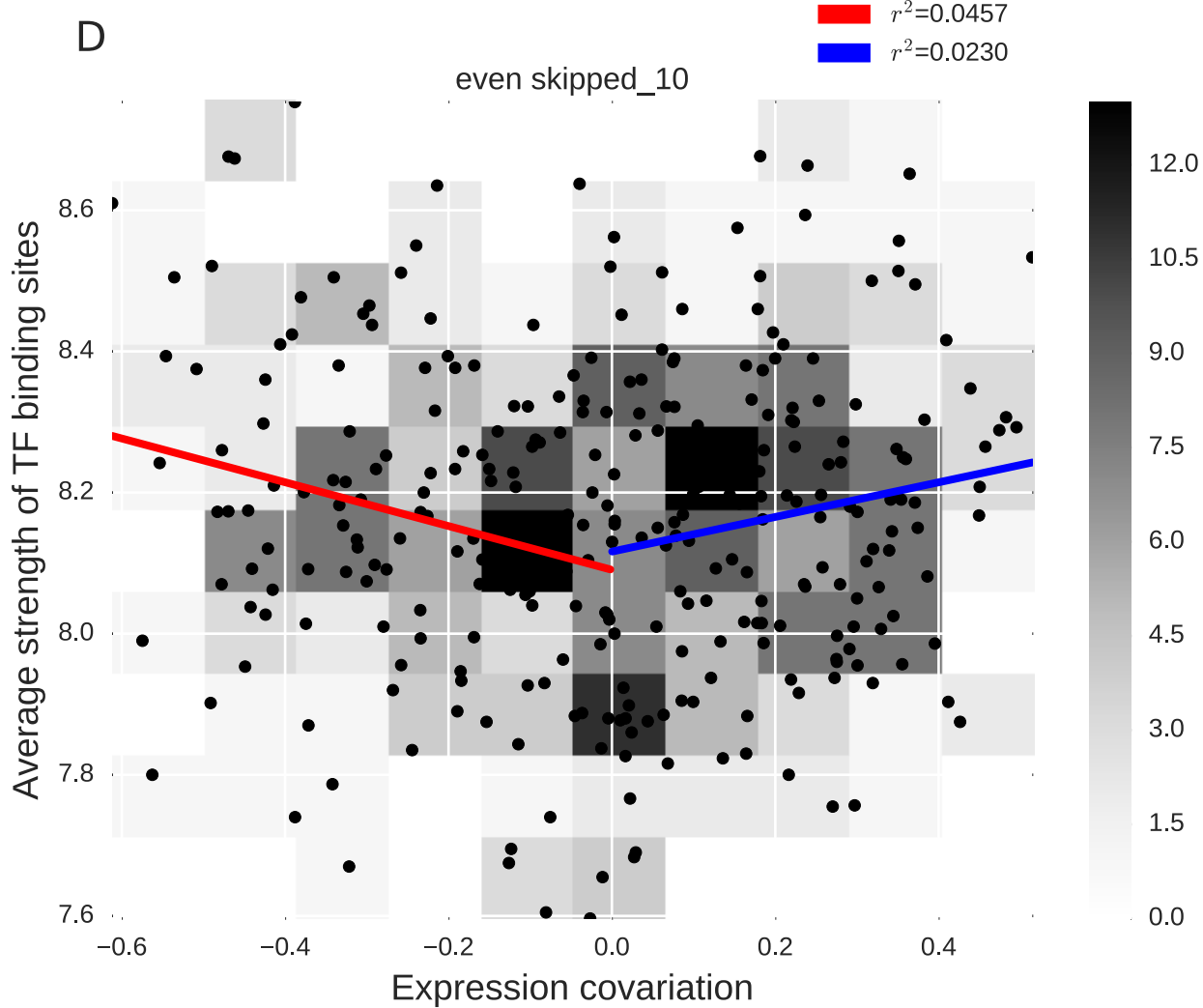
B

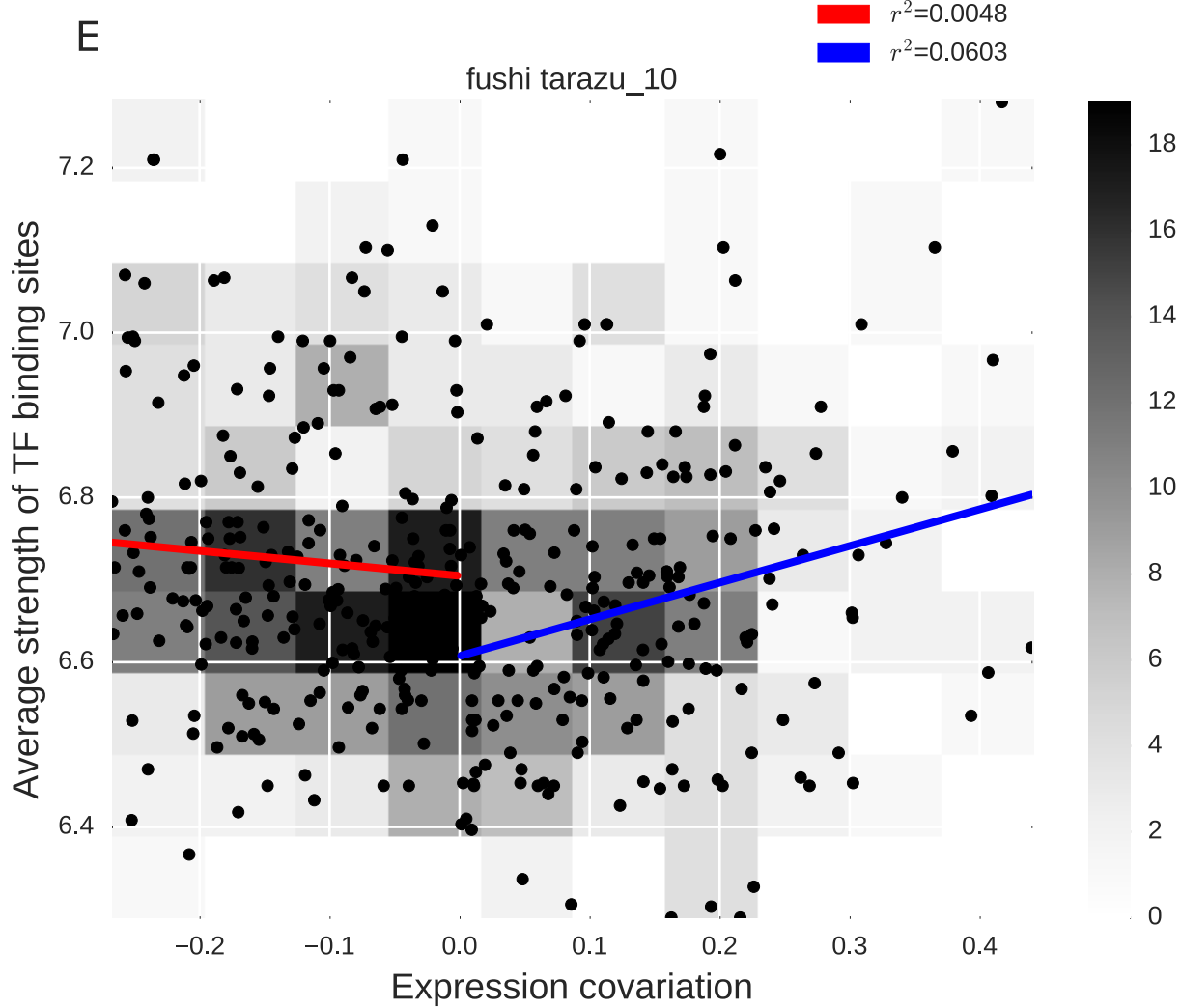


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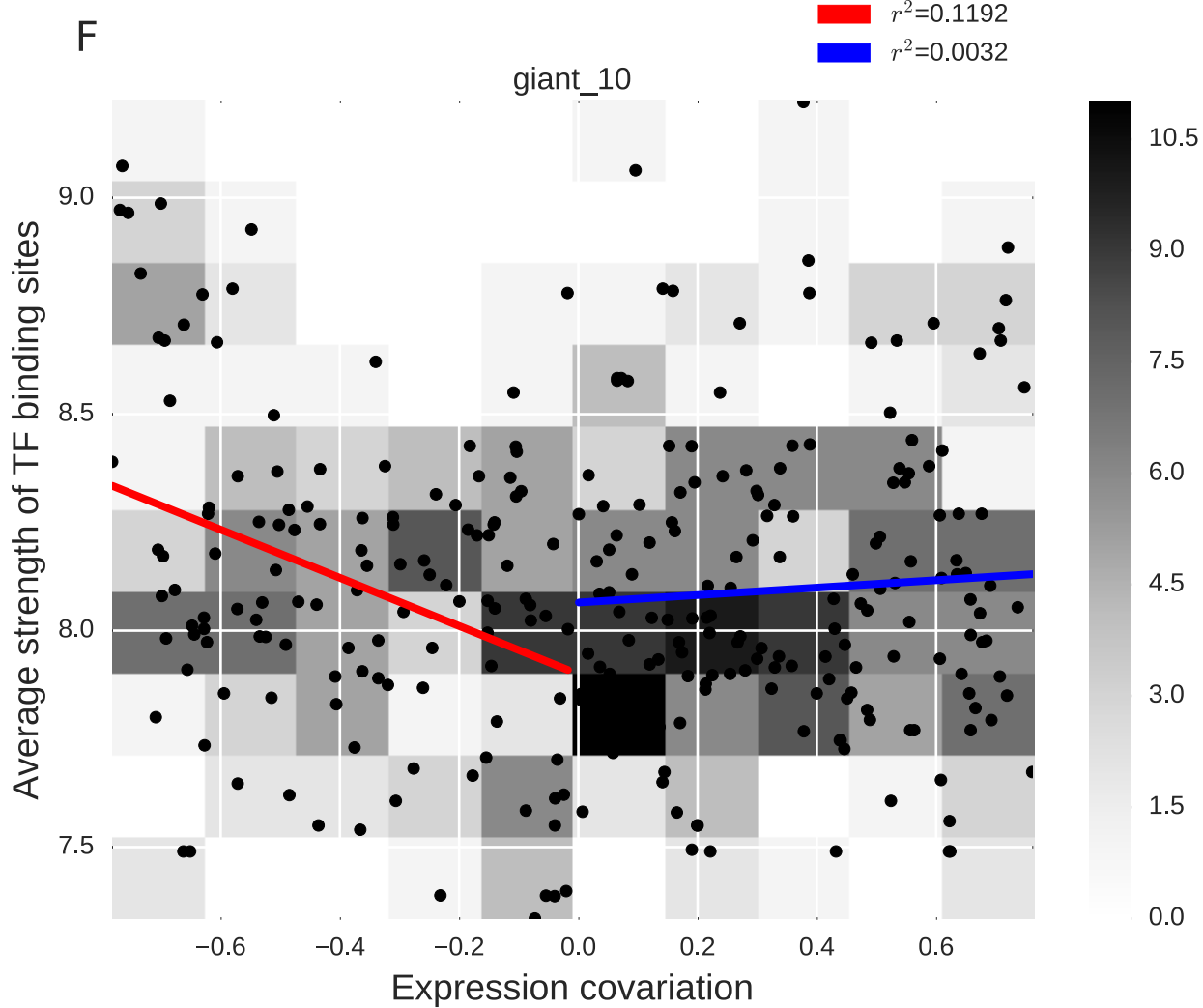


D



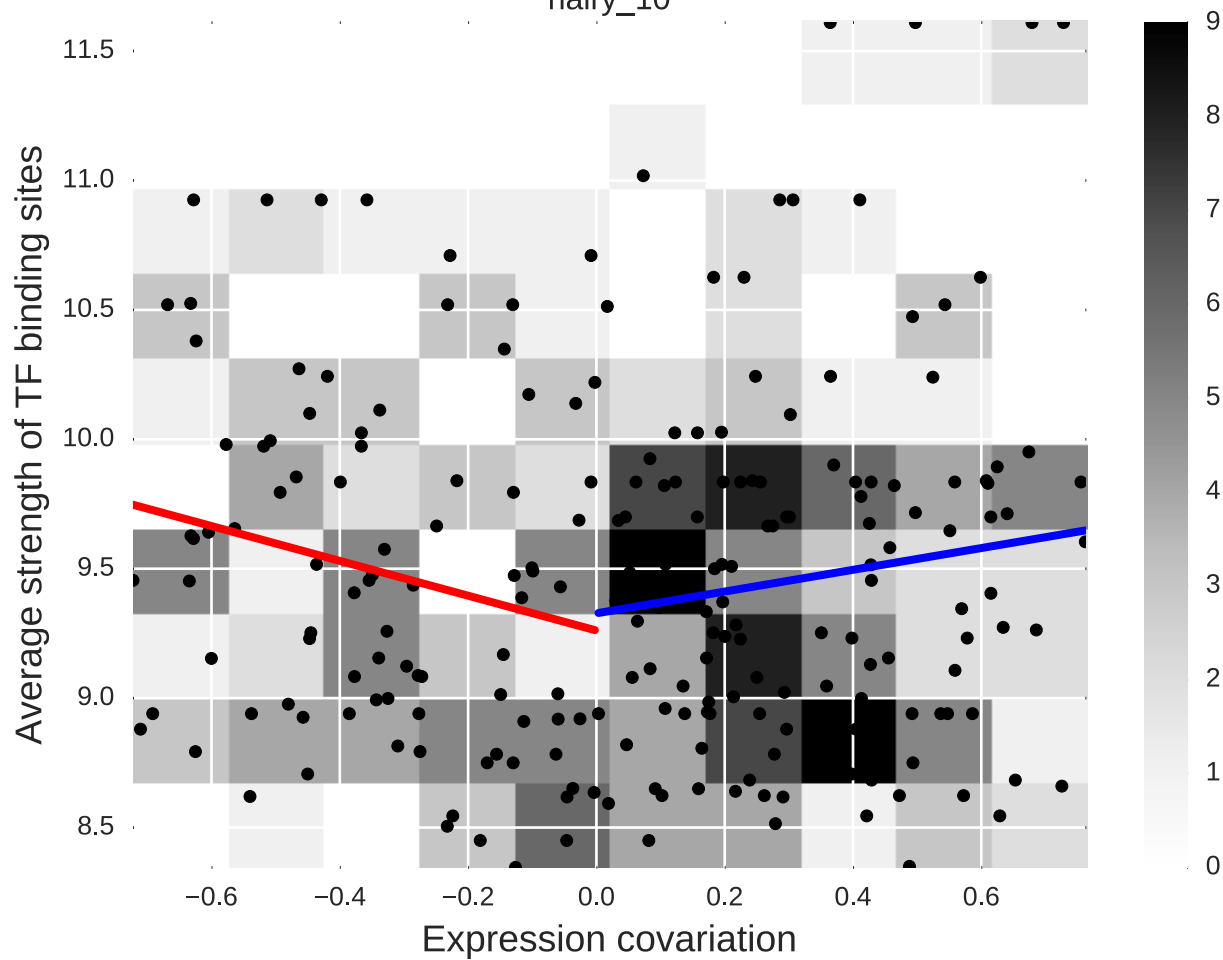


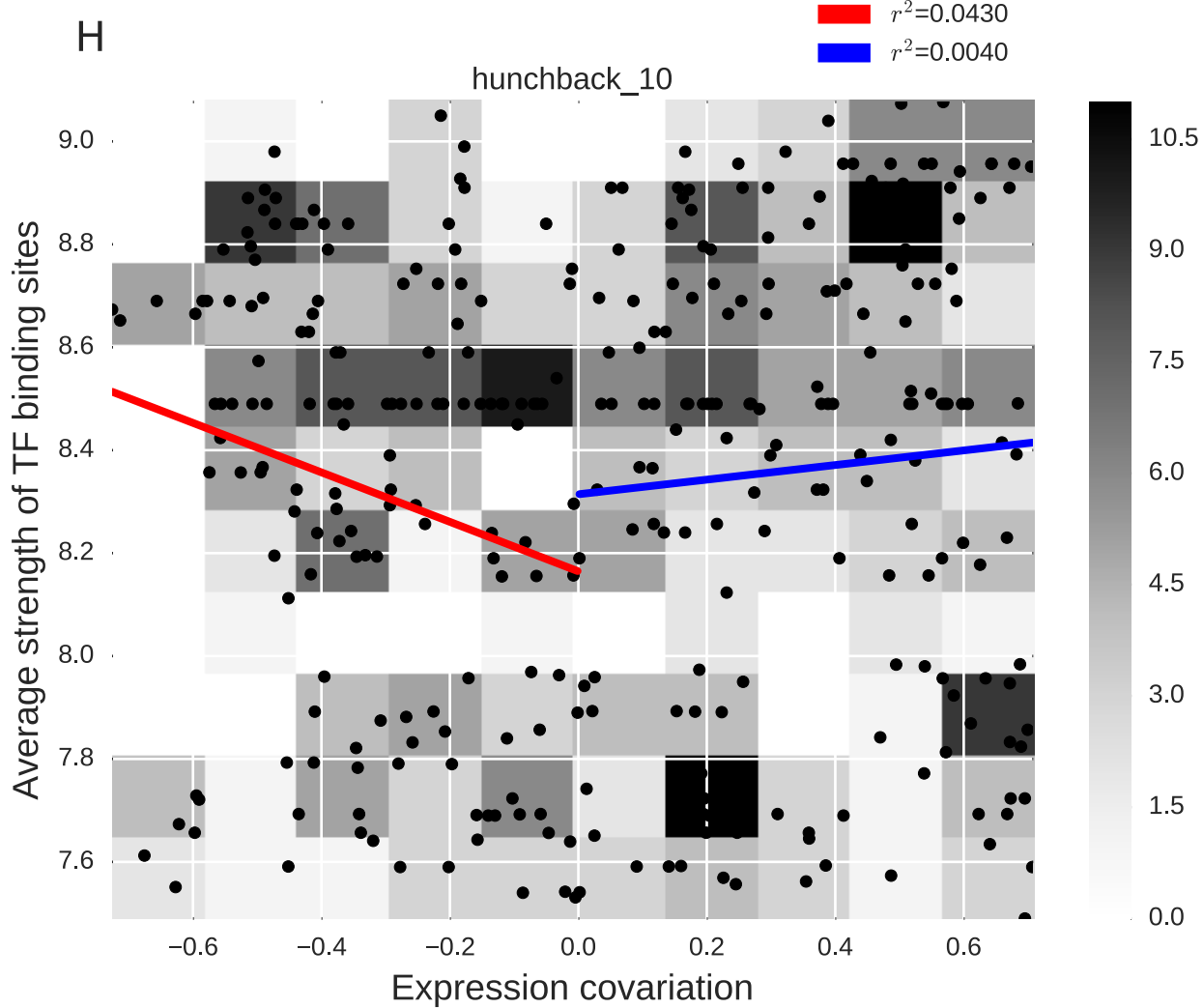
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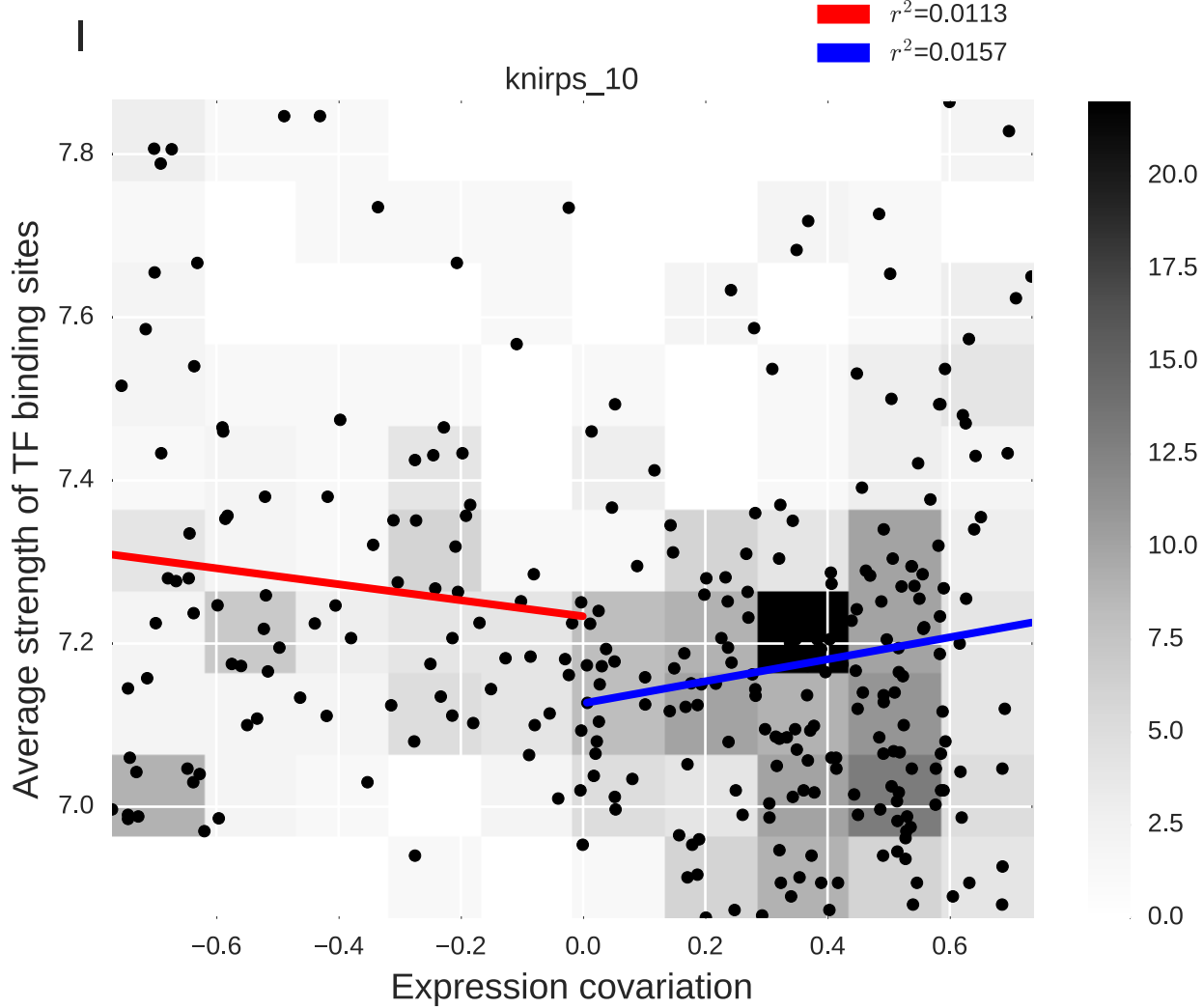


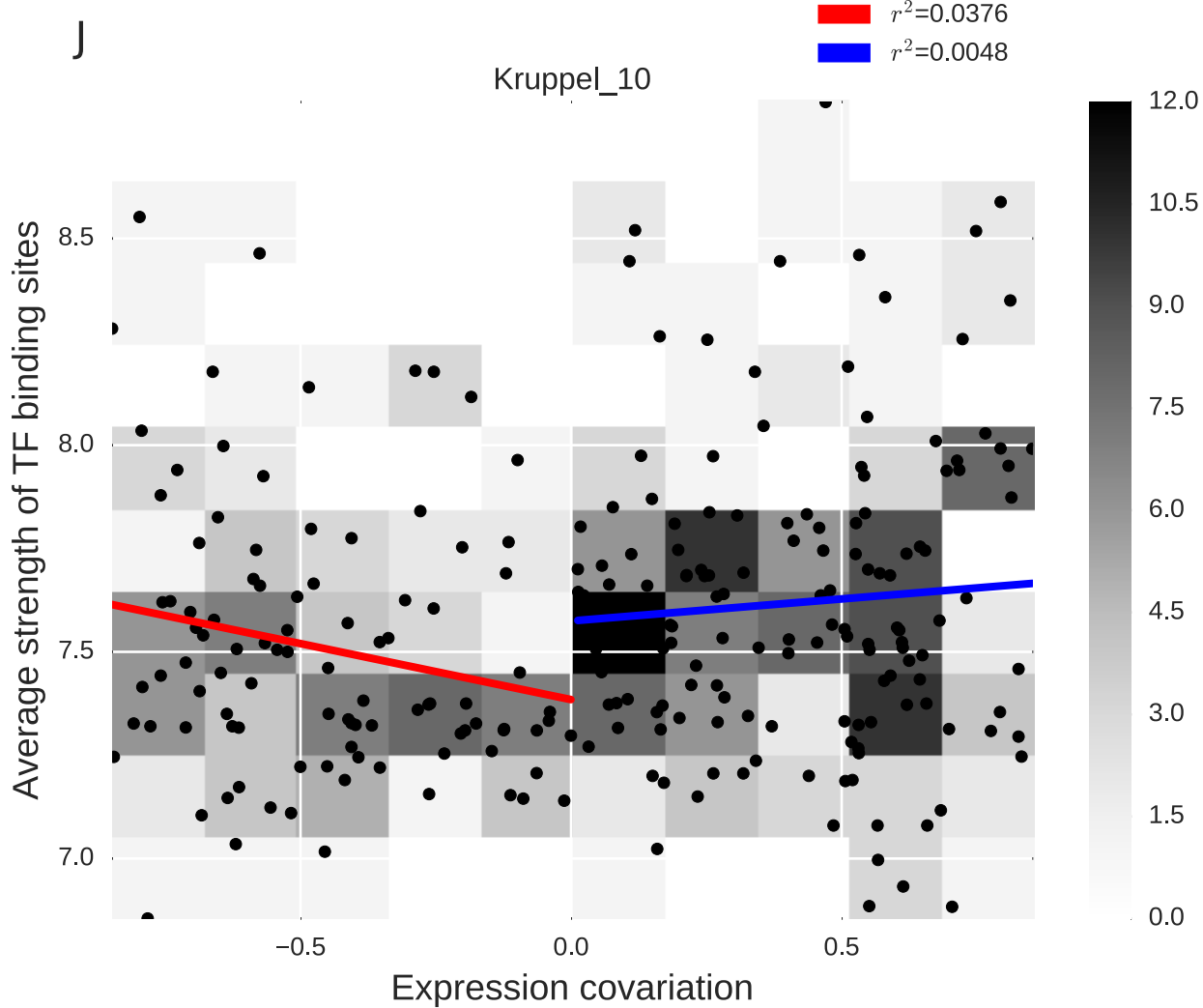
G

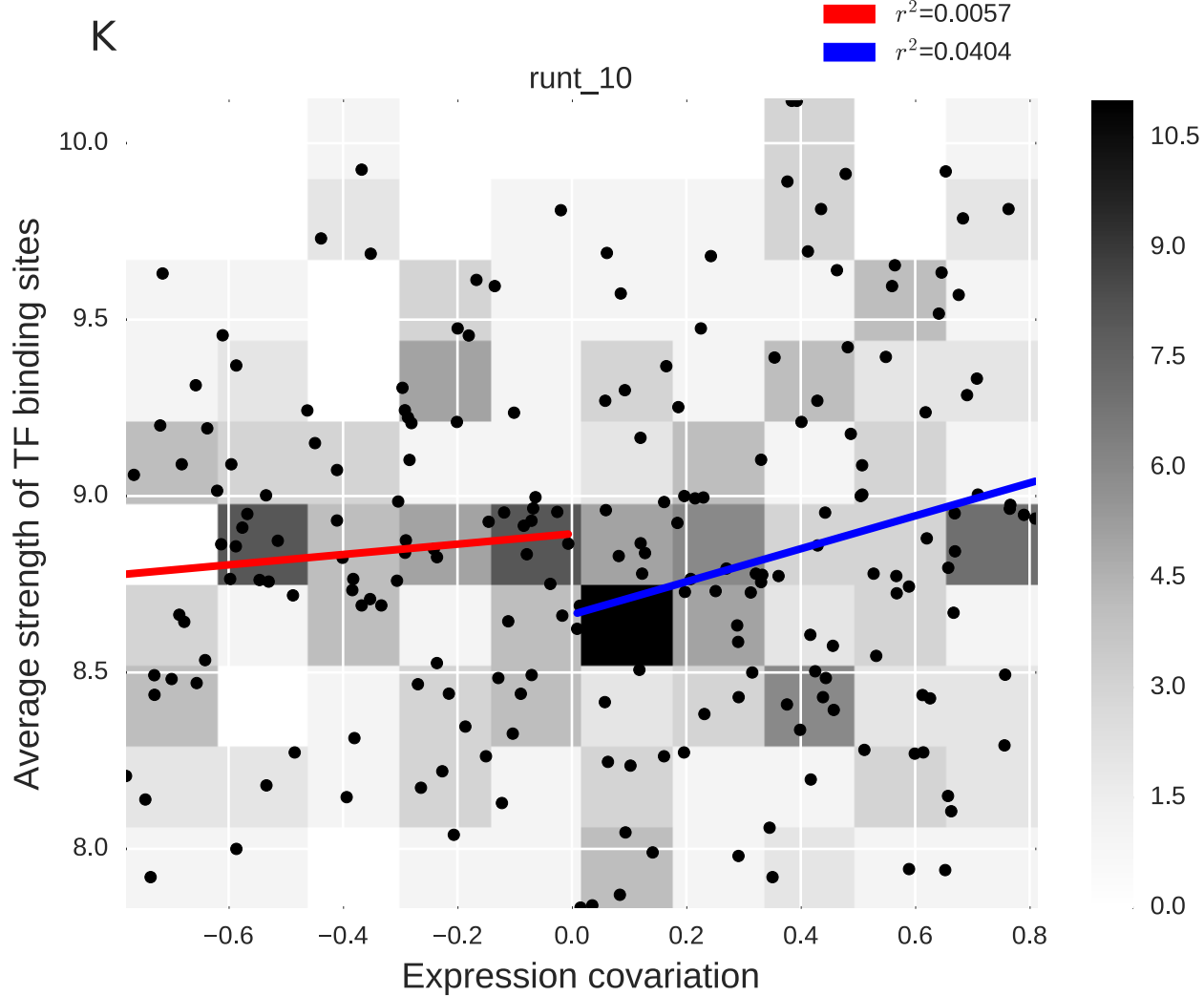
hairy\_10

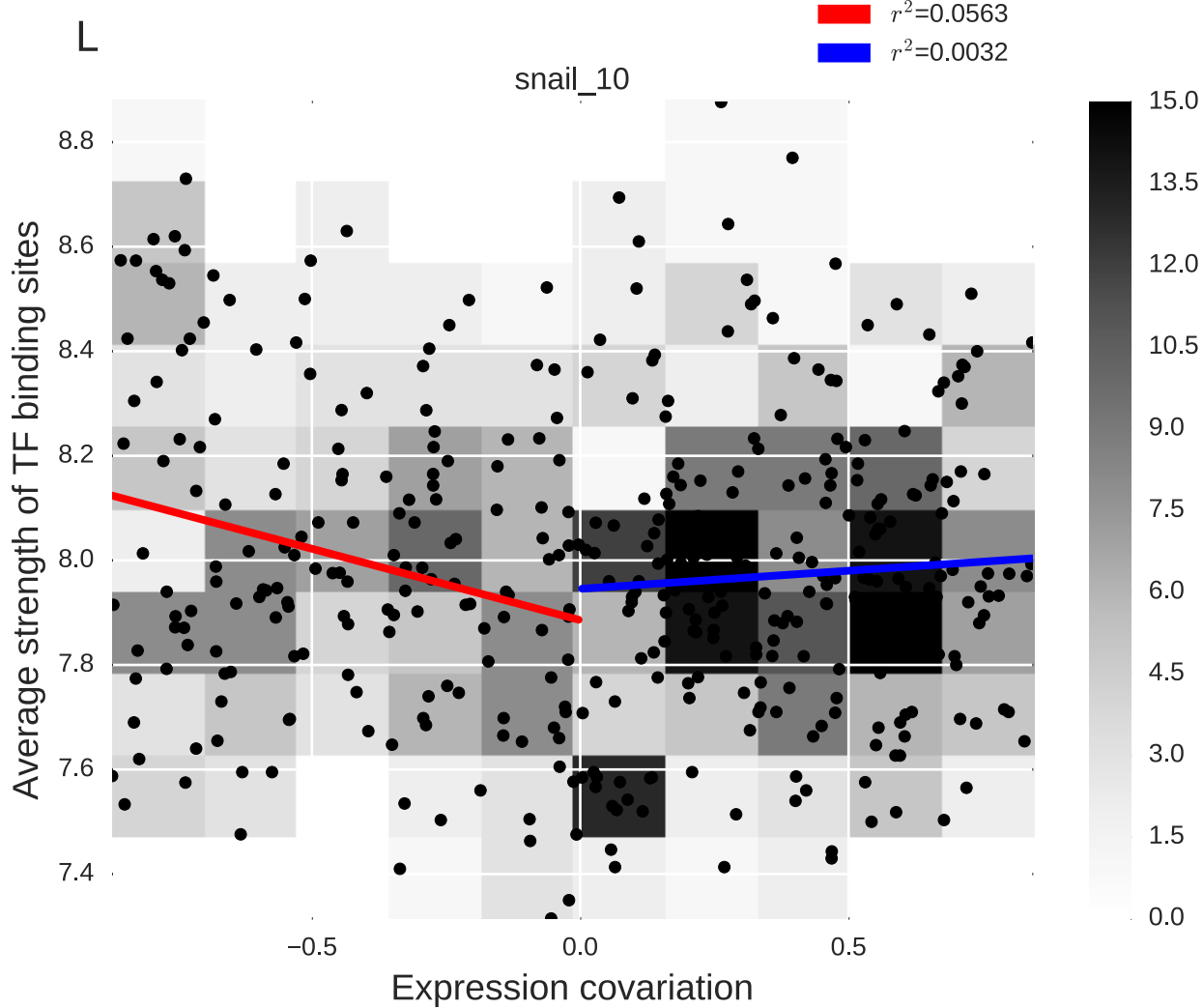
 $r^2=0.0424$   
 $r^2=0.0144$ 



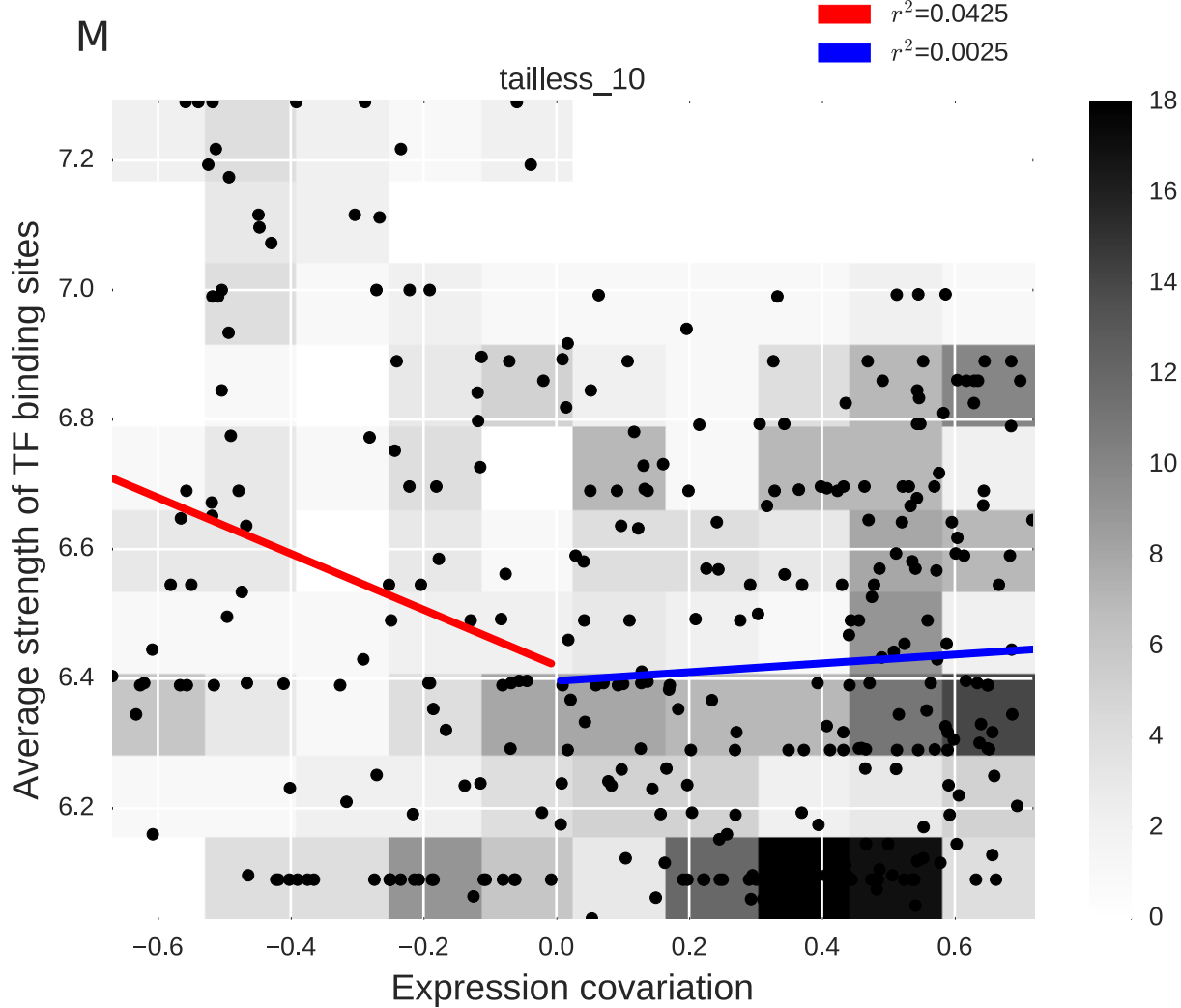




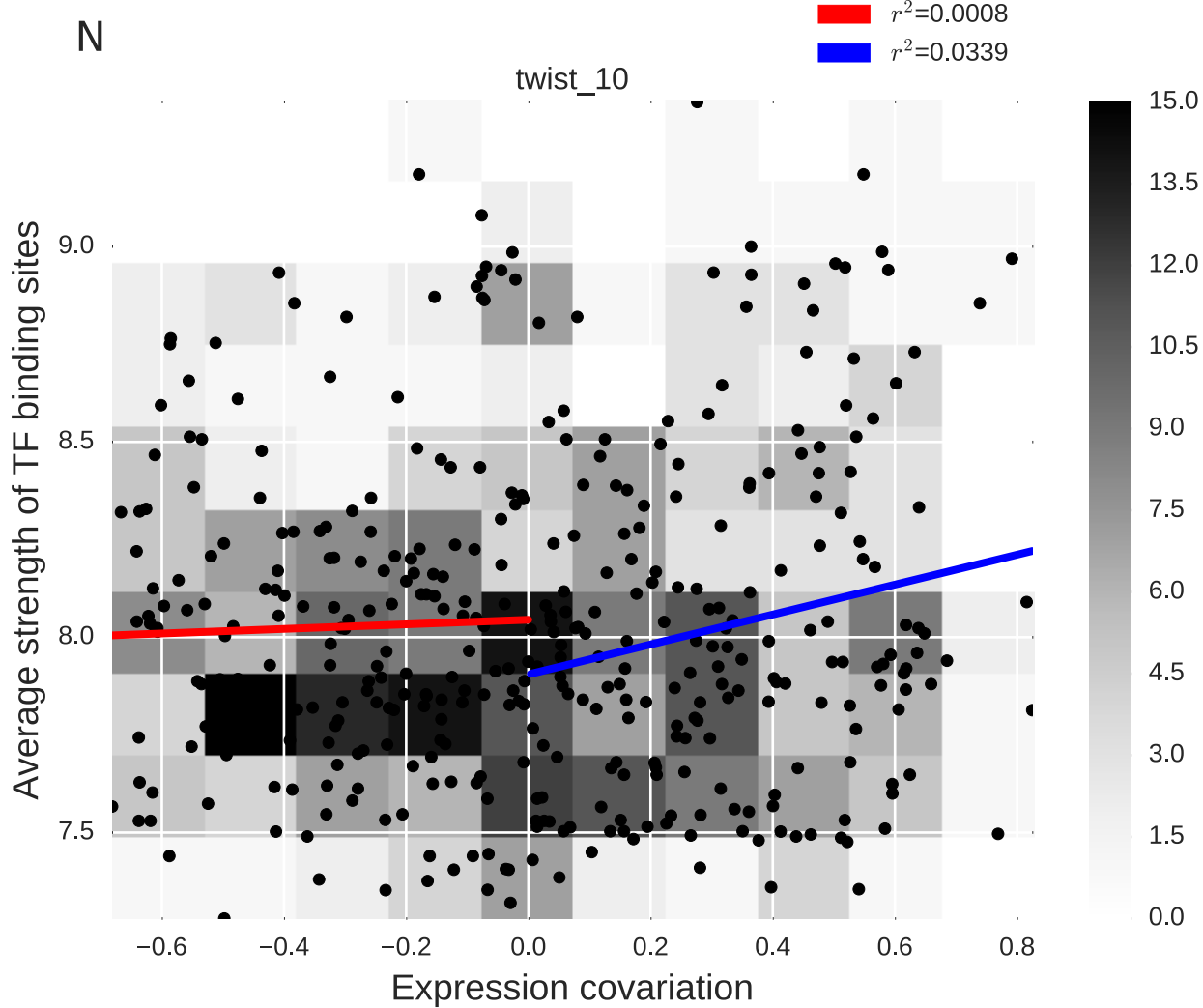




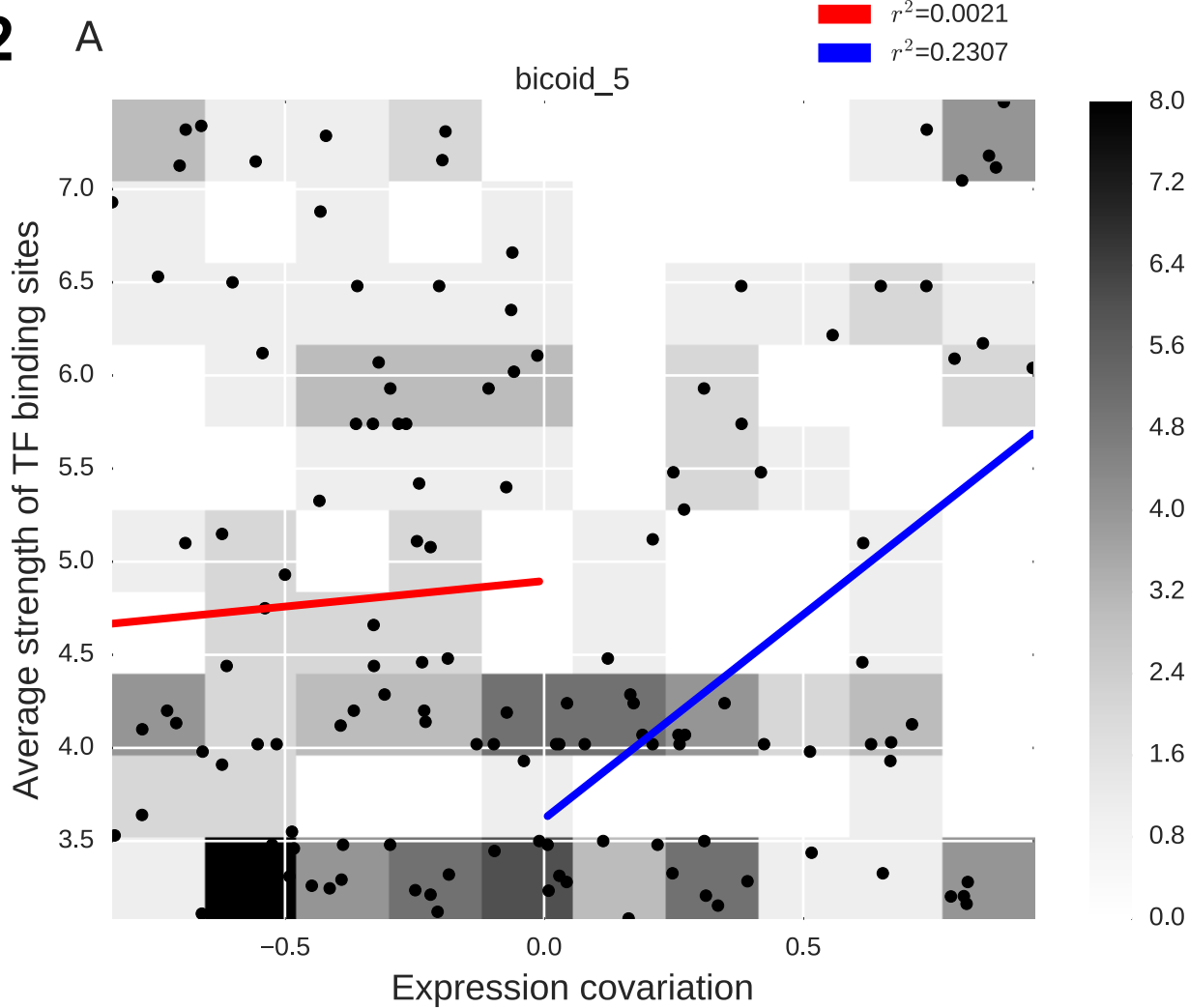
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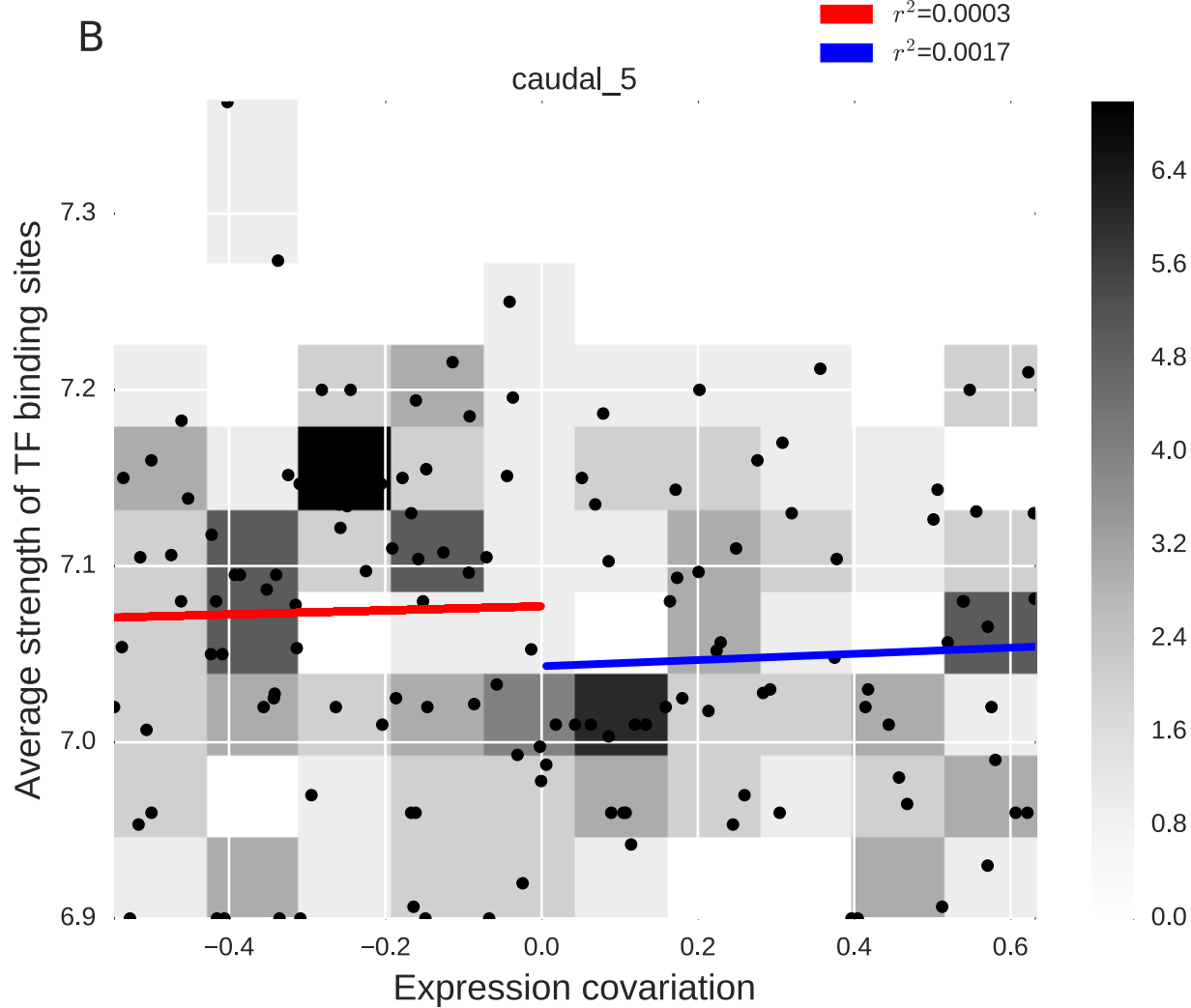


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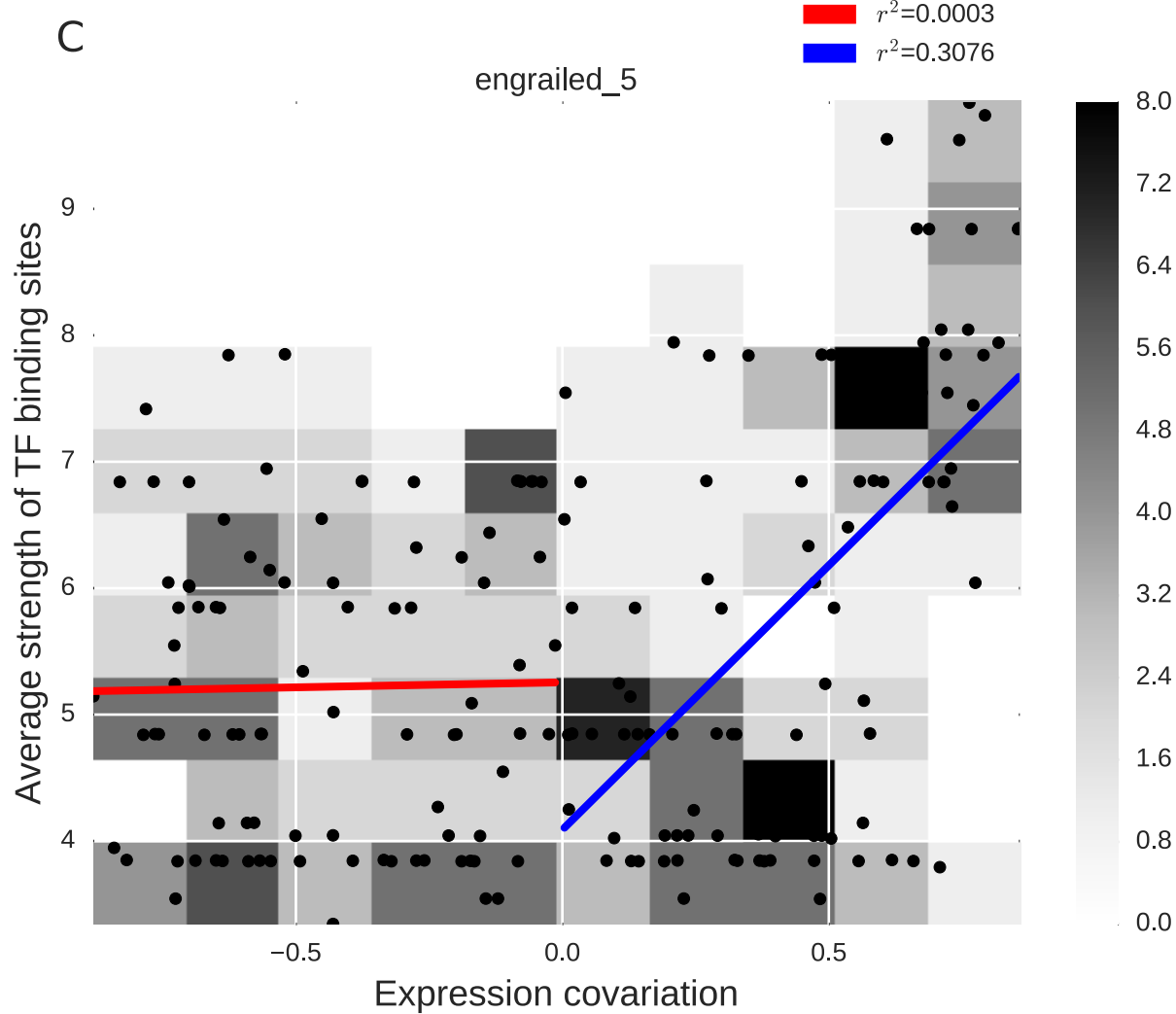


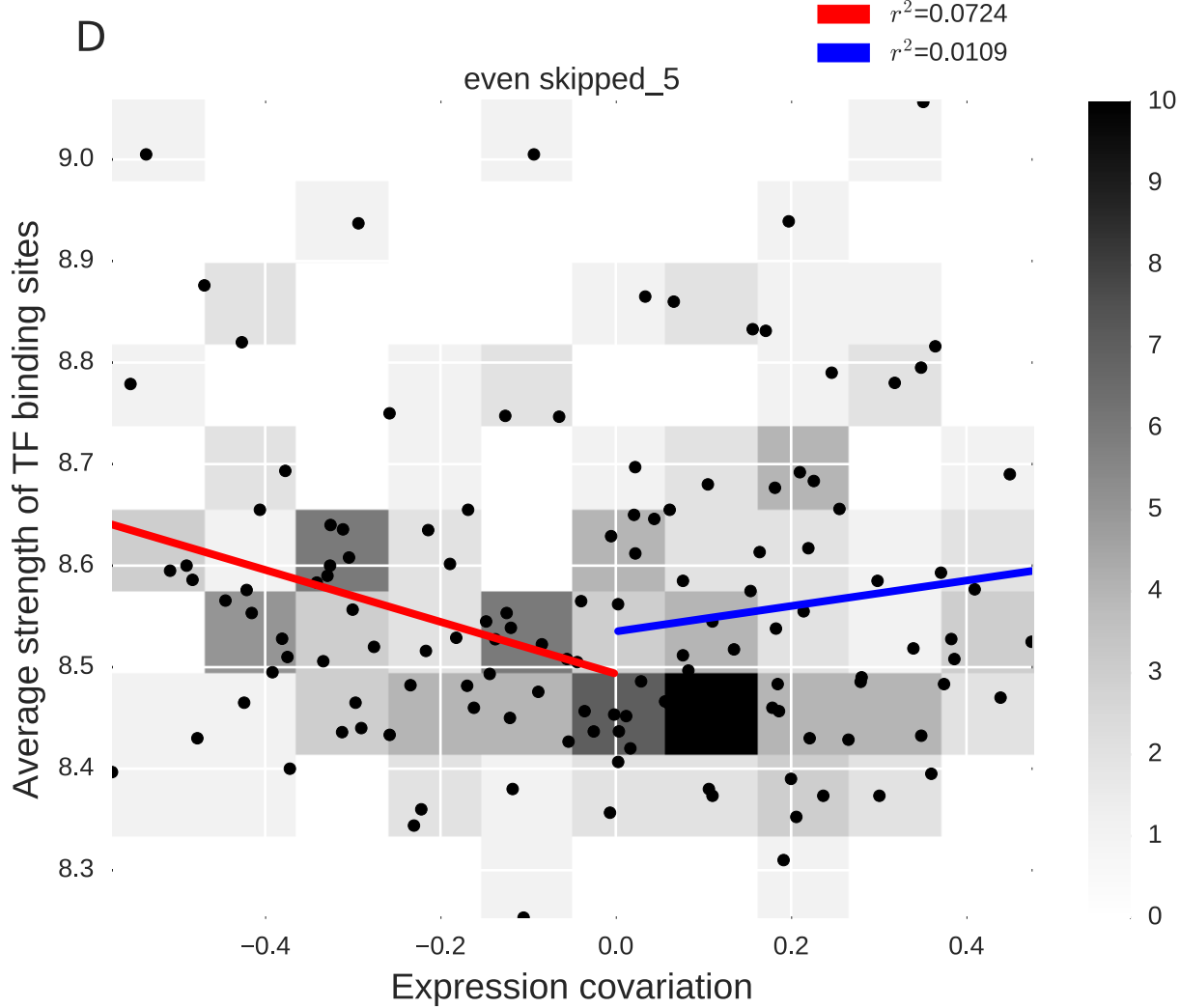
**Figure S2:** The relationship between regulatory strength and average TF binding site strength for target genes using the top 5% of binding scores for each TF. Here we plot the relationship between regulatory strength and average TF binding strength for all our TFs of interest. Each black dot represents a target for the TF in the panel with at least three binding motifs. The x-axis shows the correlations between the expression level of each TF and its target genes across all our samples. The y-axis shows the average TF binding site strength for binding sites located in the assumed region of regulatory DNA for each target gene. The blue and red lines show the linear regression for the positively and negatively correlated target genes, respectively, and  $r^2$  values are displayed for these best fit lines.

**S2****A**



C

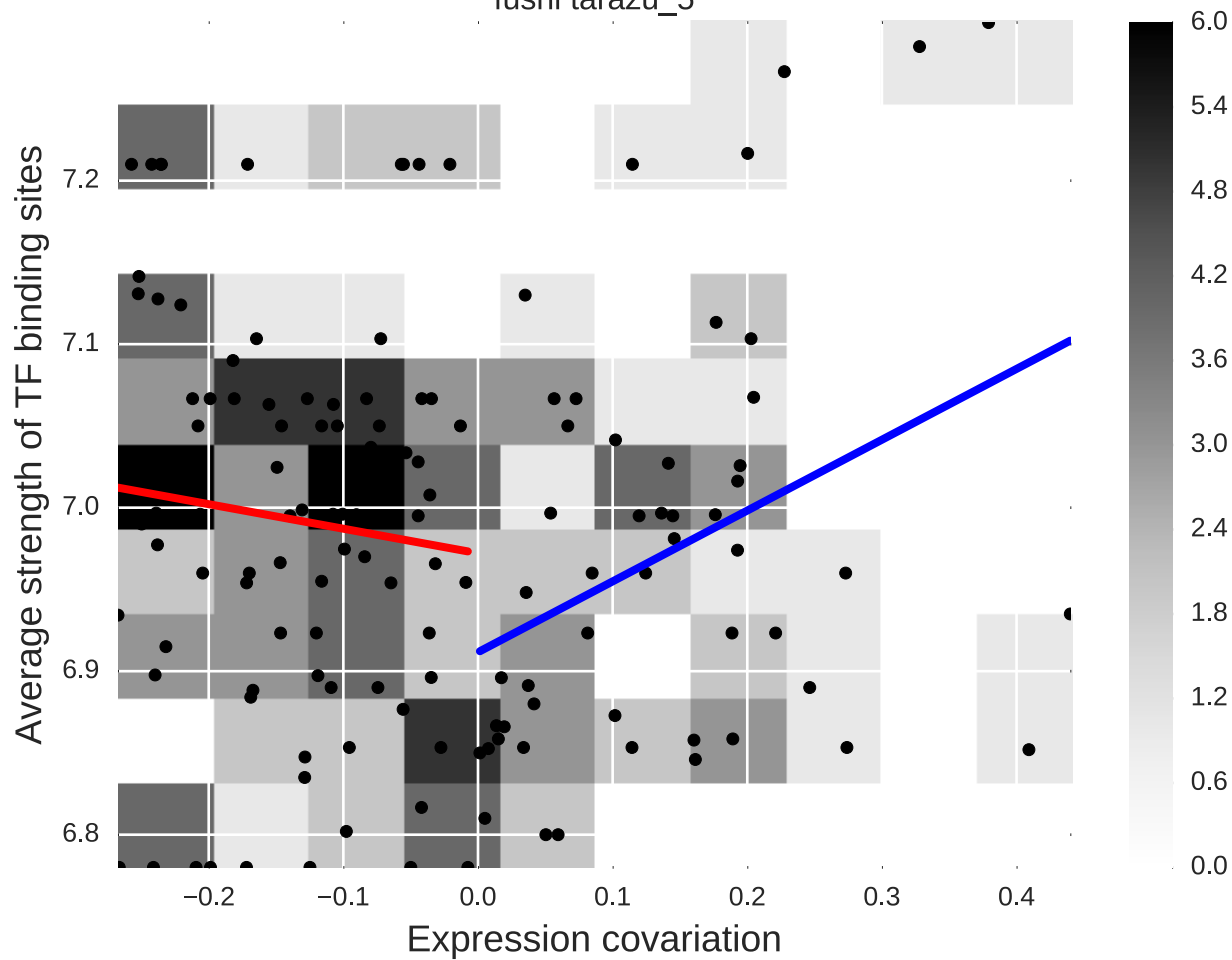


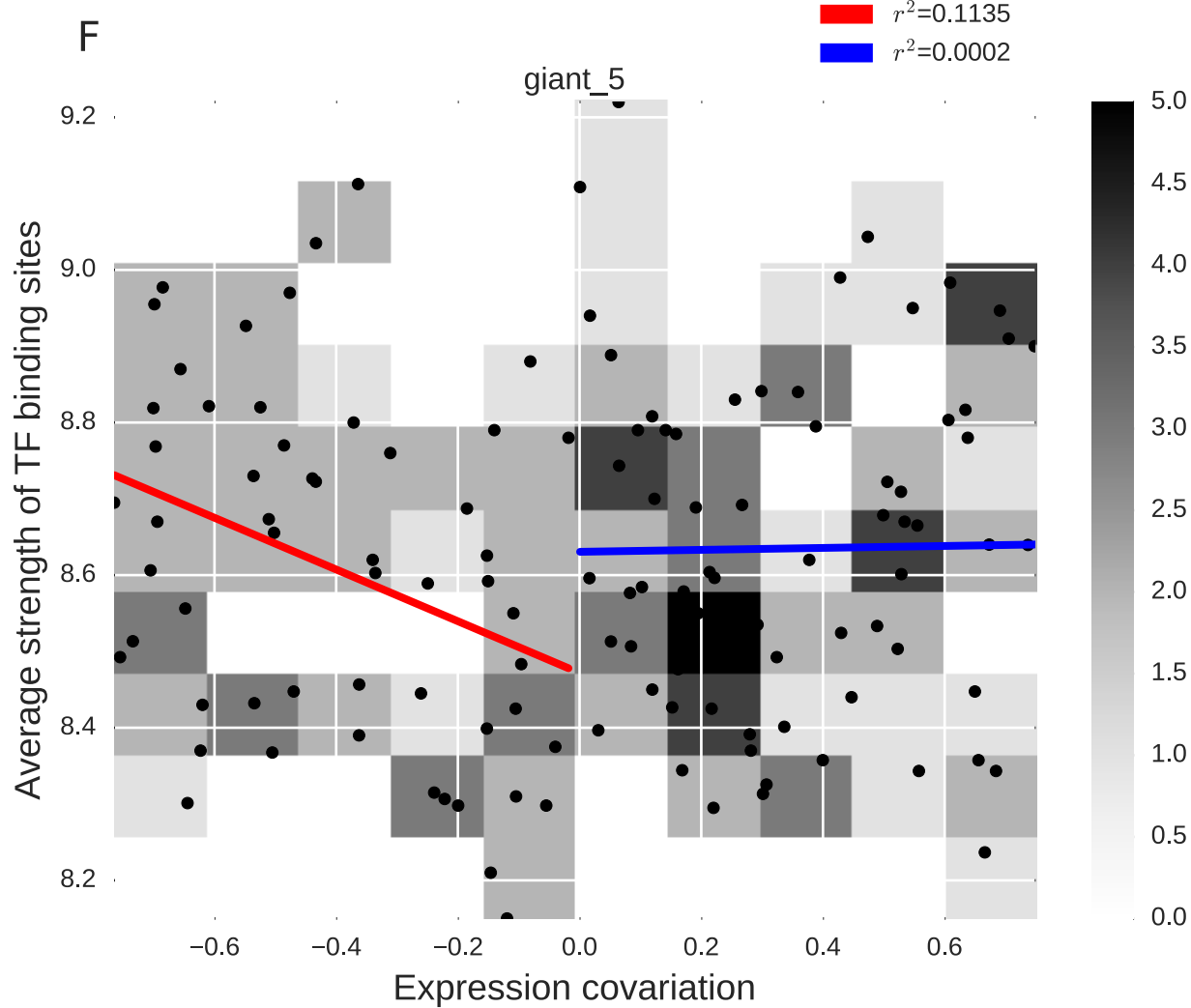


E

fushi tarazu\_5

$r^2=0.0093$   
 $r^2=0.1306$





G

hairy\_5

 $r^2=0.0881$   
 $r^2=0.0034$ 

Average strength of TF binding sites

11.5

11.0

10.5

10.0

9.5

9.0

Expression covariation

6.4

5.6

4.8

4.0

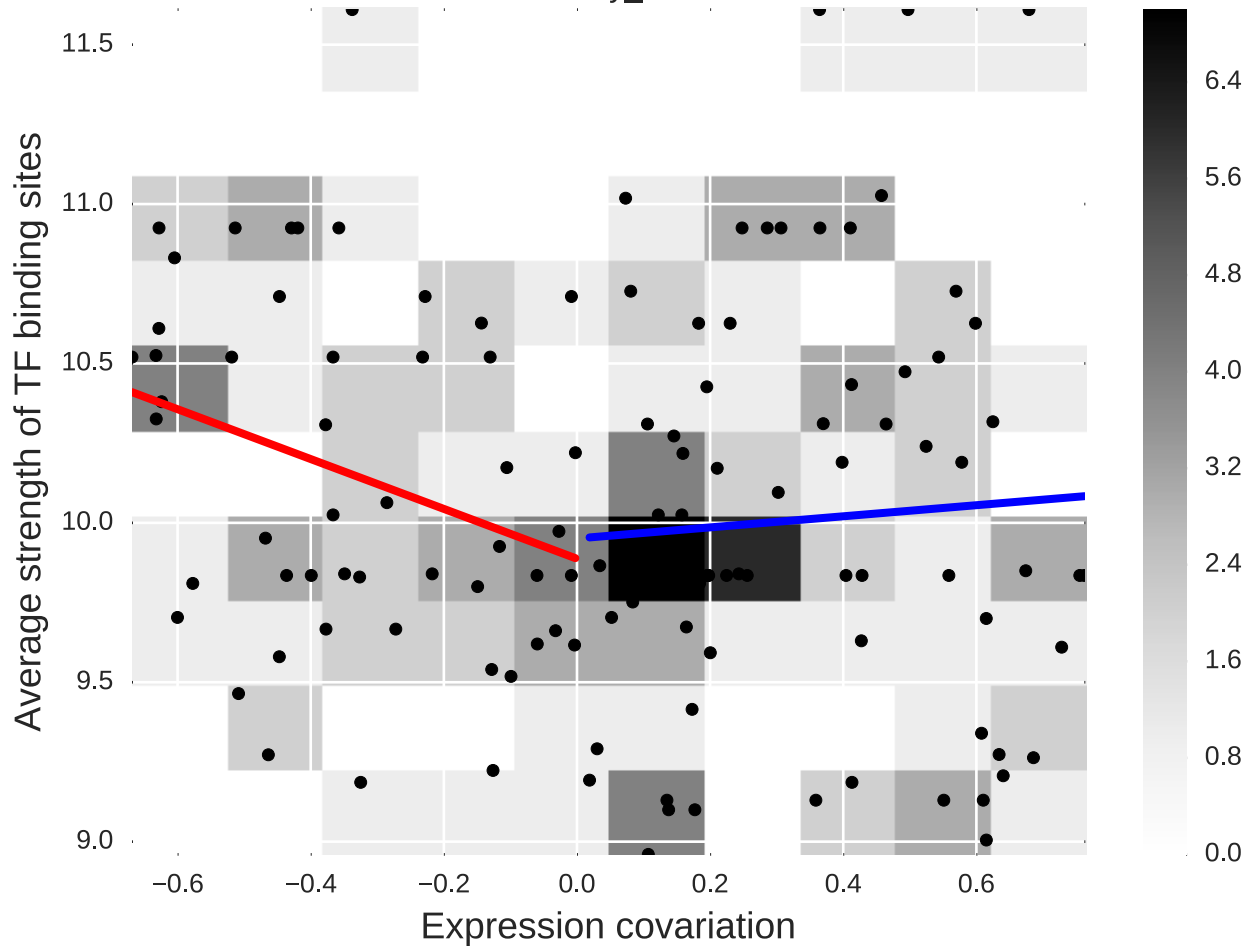
3.2

2.4

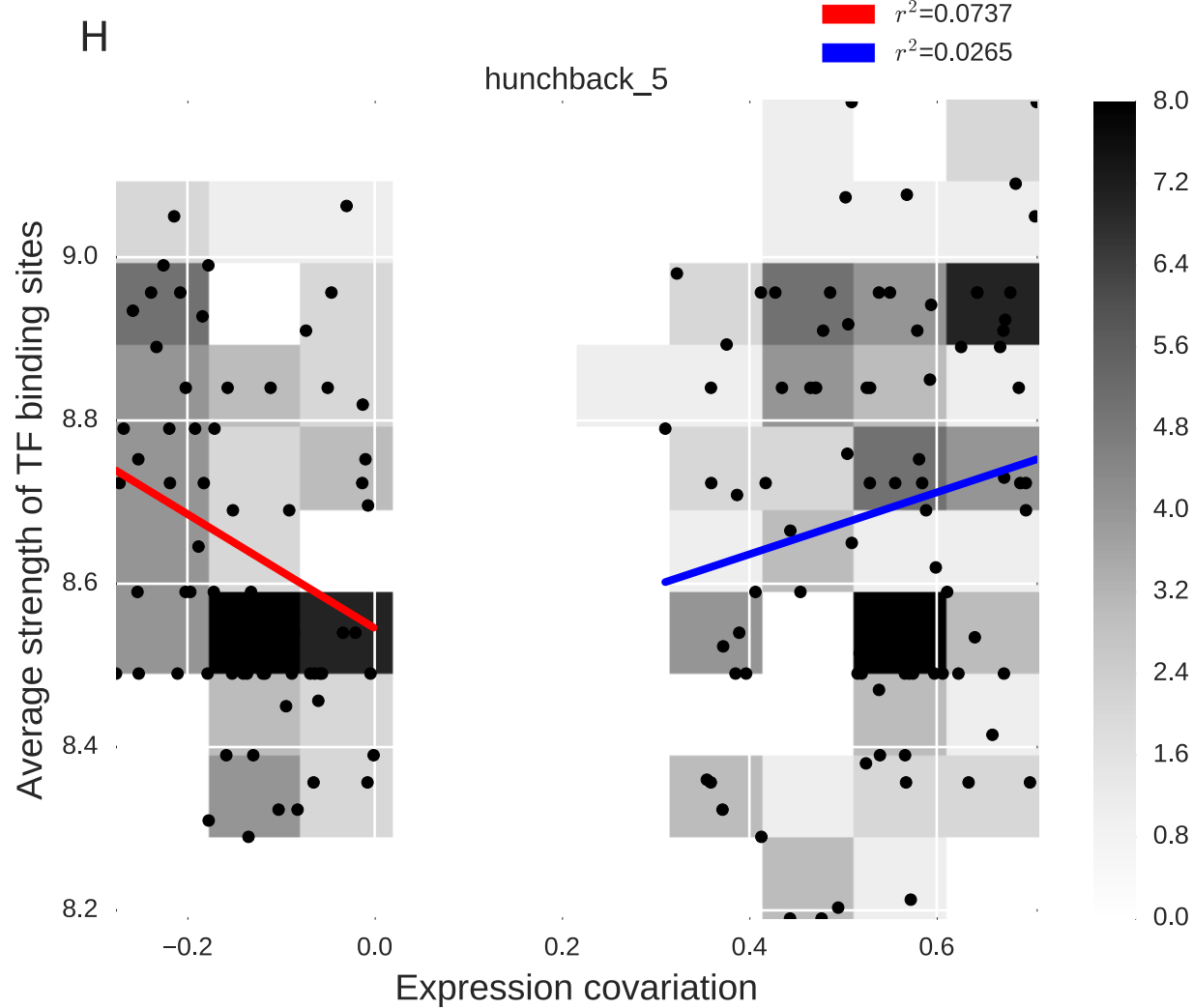
1.6

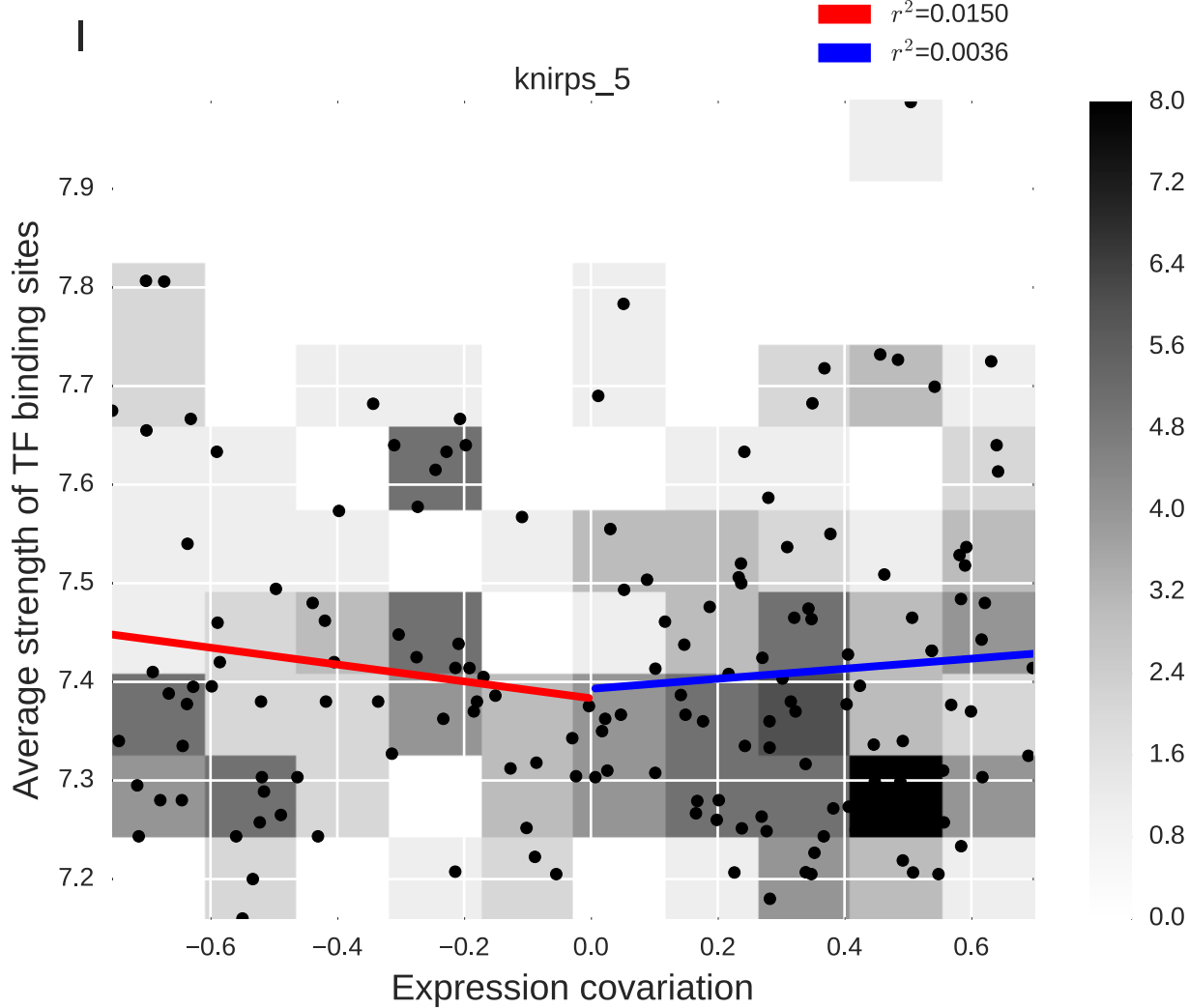
0.8

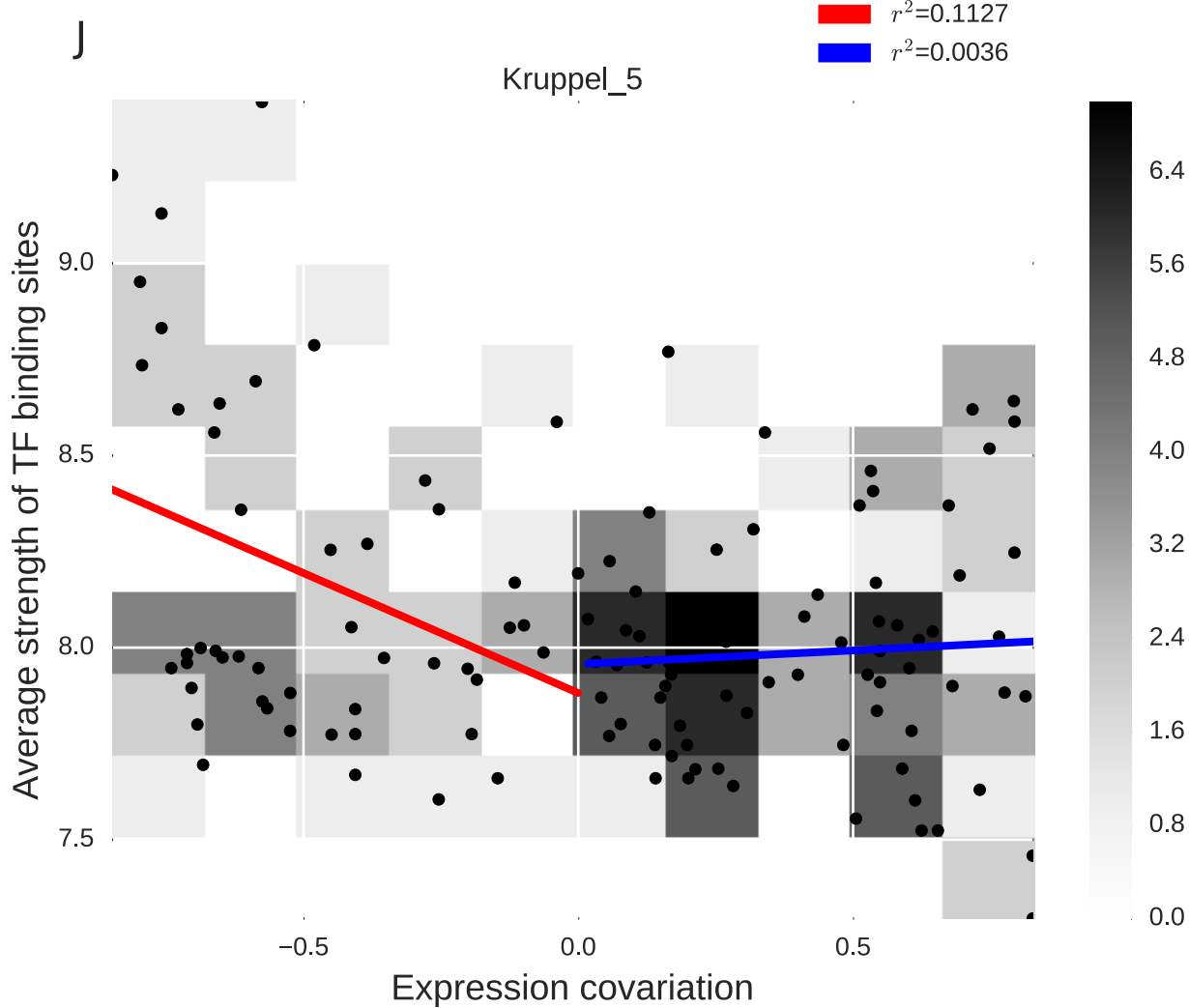
0.0

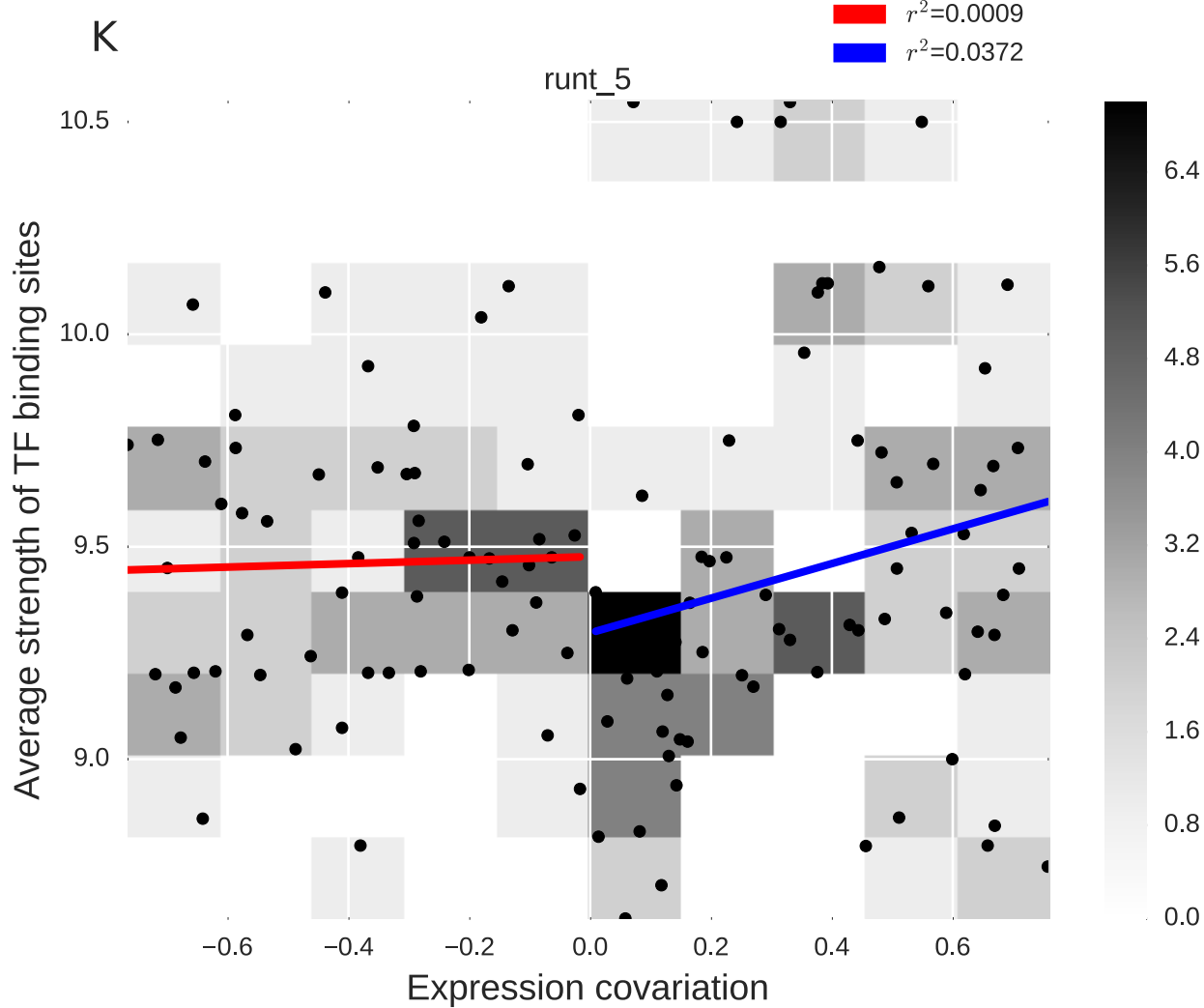


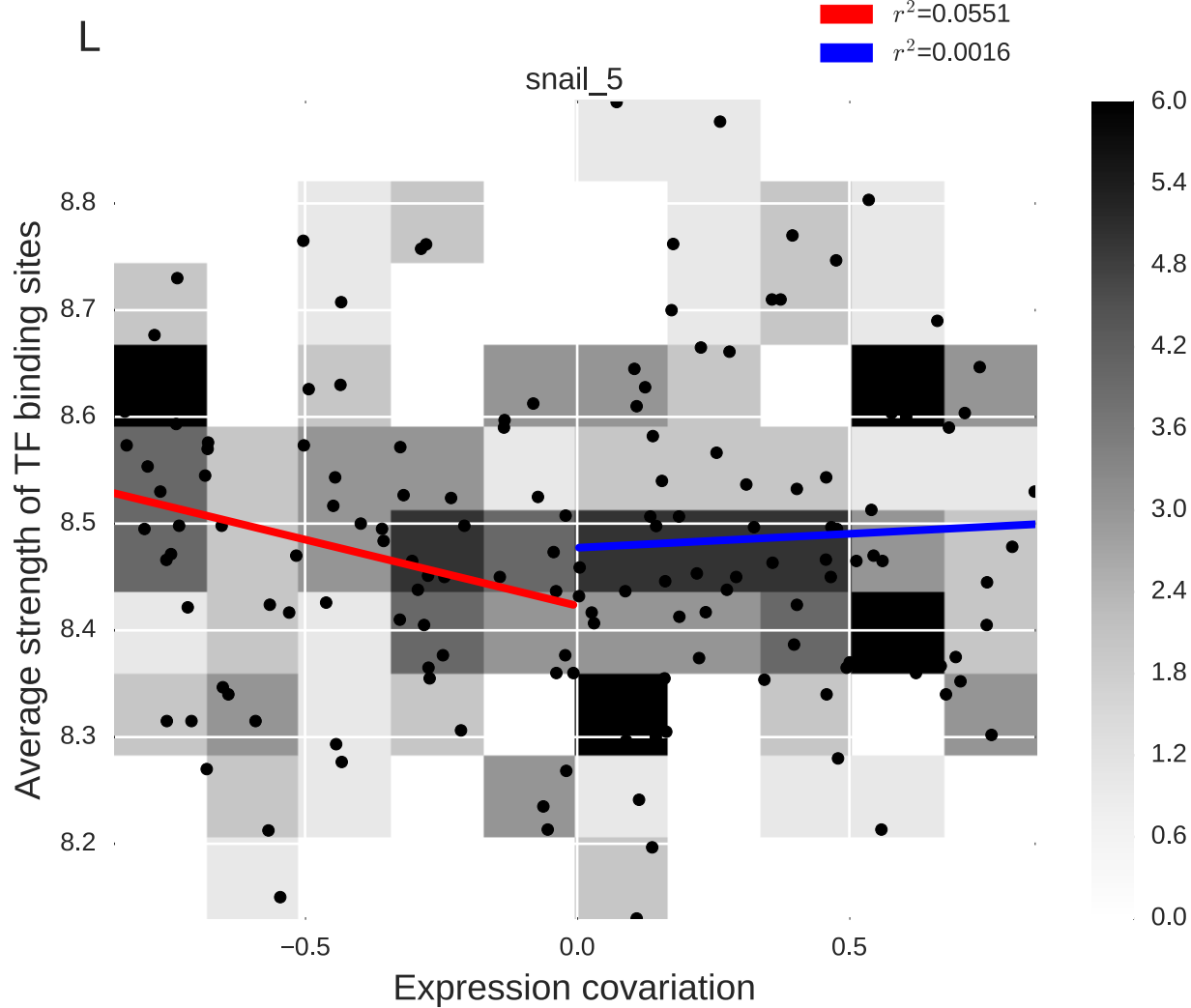
H

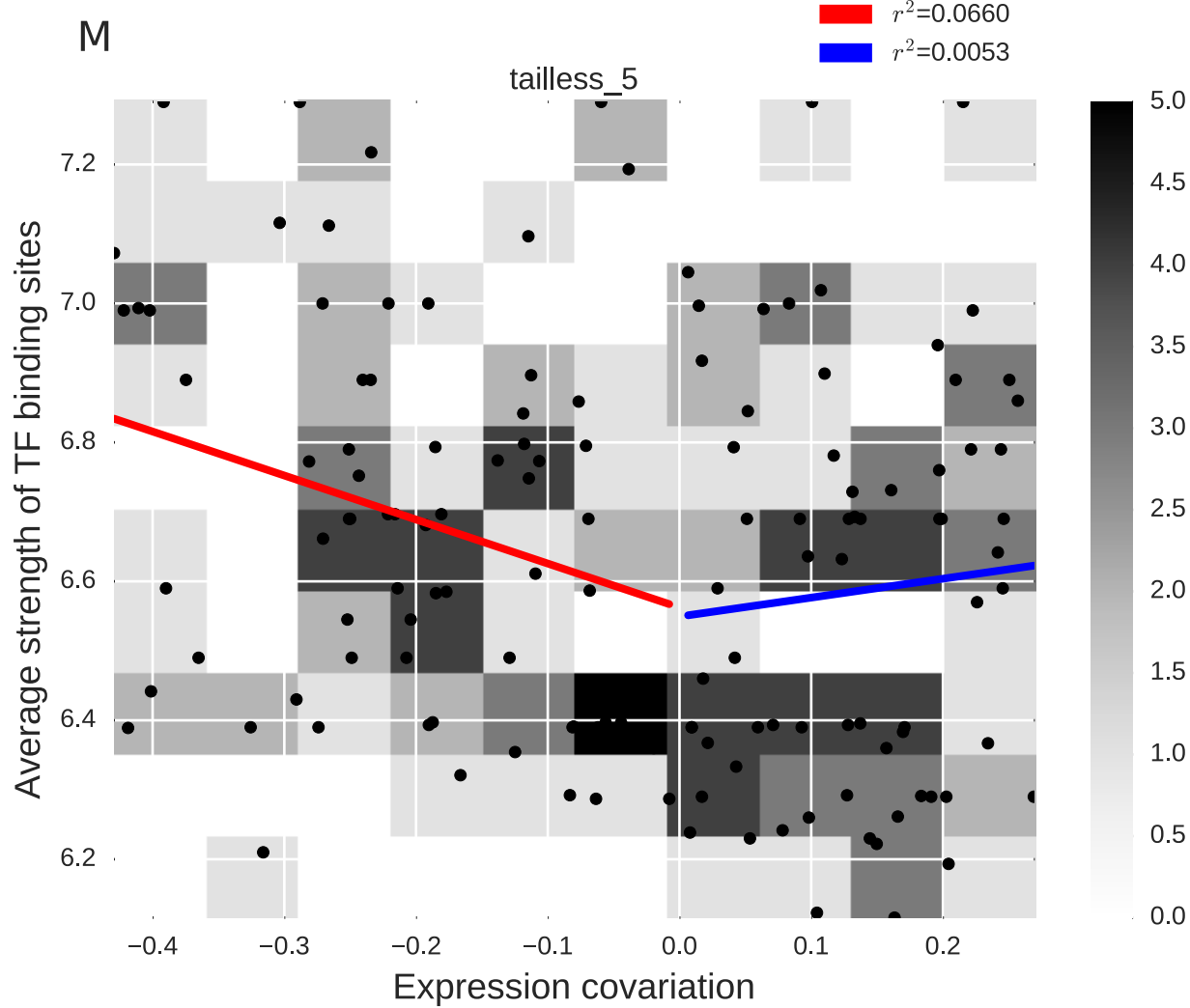


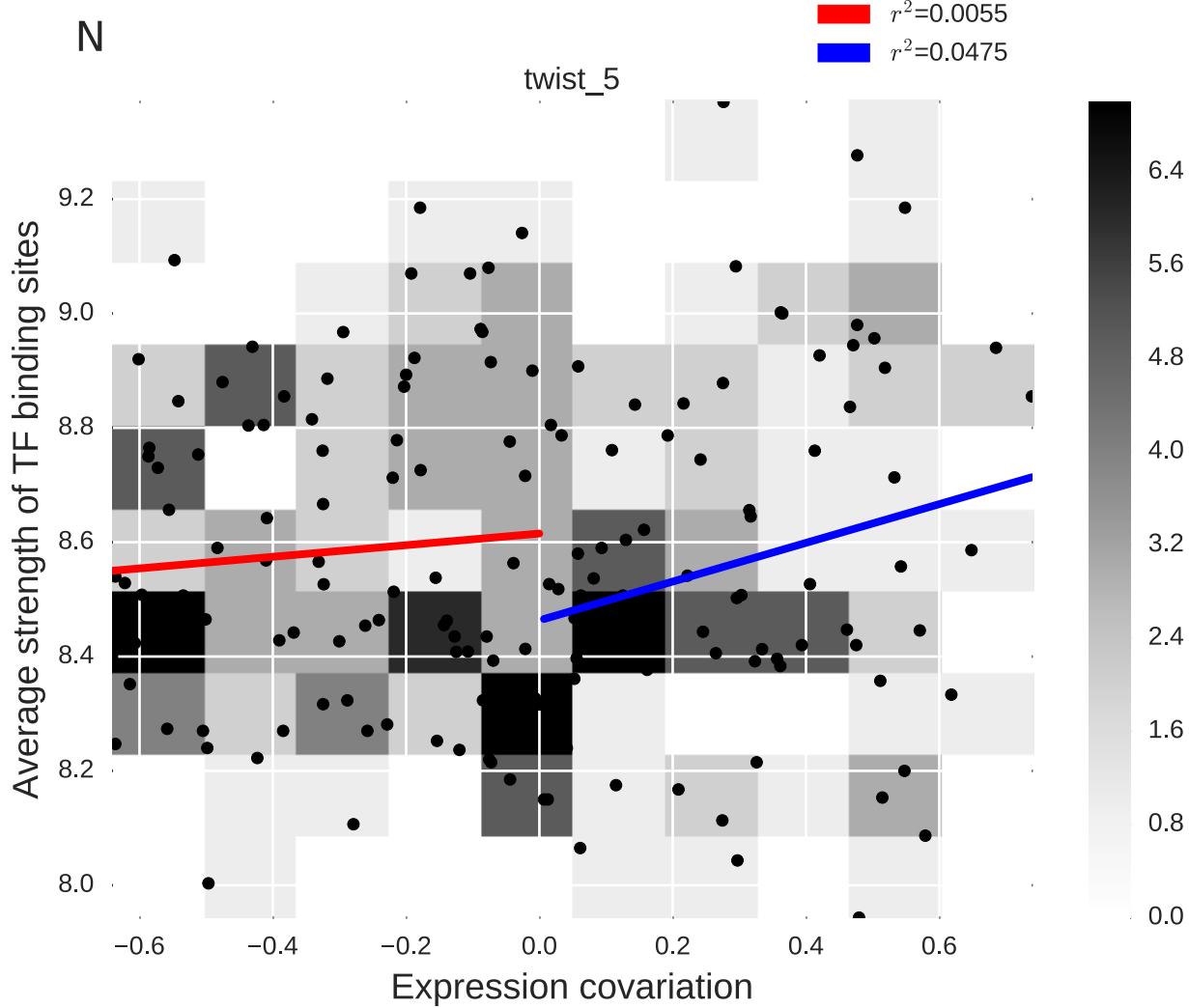








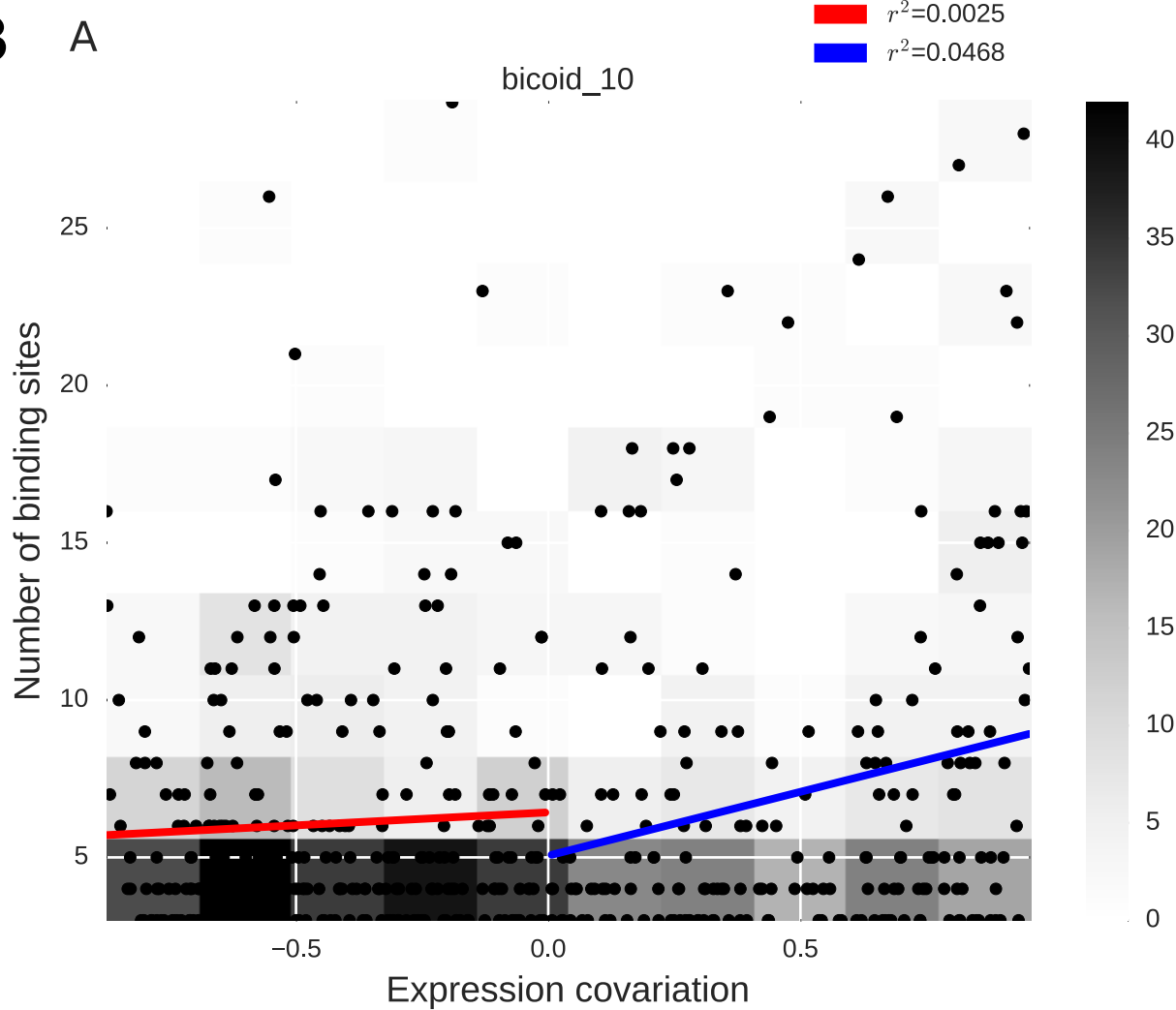


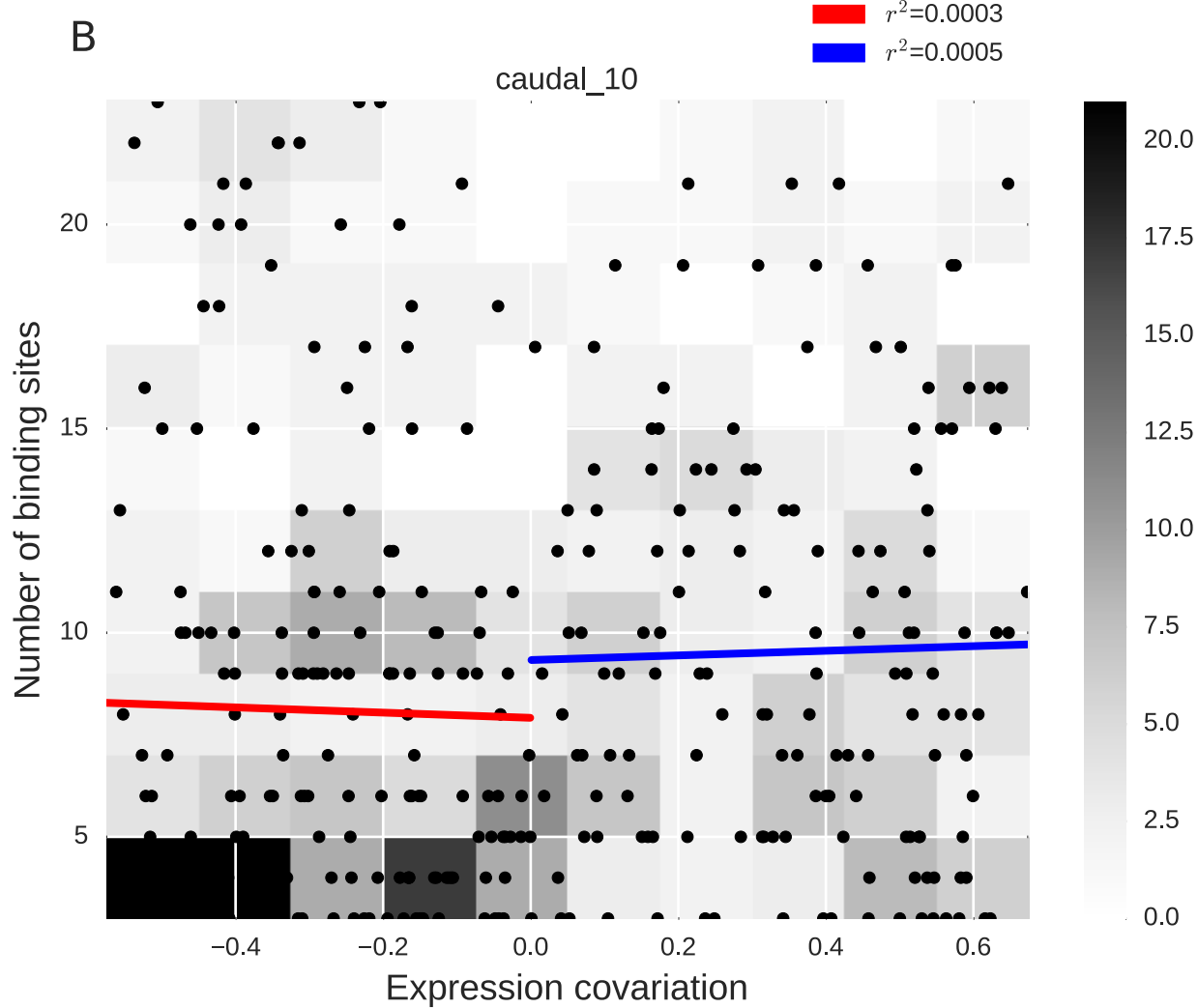


**Figure S3:** The relationship between regulatory strength and number of TF binding sites for target genes using the top 10% of binding scores for each TF. Here we plot the relationship between regulatory strength and number of TF binding sites for all our TFs of interest. Each black dot represents a target for the TF in the panel with at least three binding motifs. The x-axis shows the correlations between the expression level of each TF and its target genes across all our samples. The y-axis shows the number of TF binding sites which located in the assumed region of regulatory DNA for each target gene. The blue and red lines show the linear regression for the positively and negatively correlated target genes, respectively, and  $r^2$  values are displayed for these best fit lines.

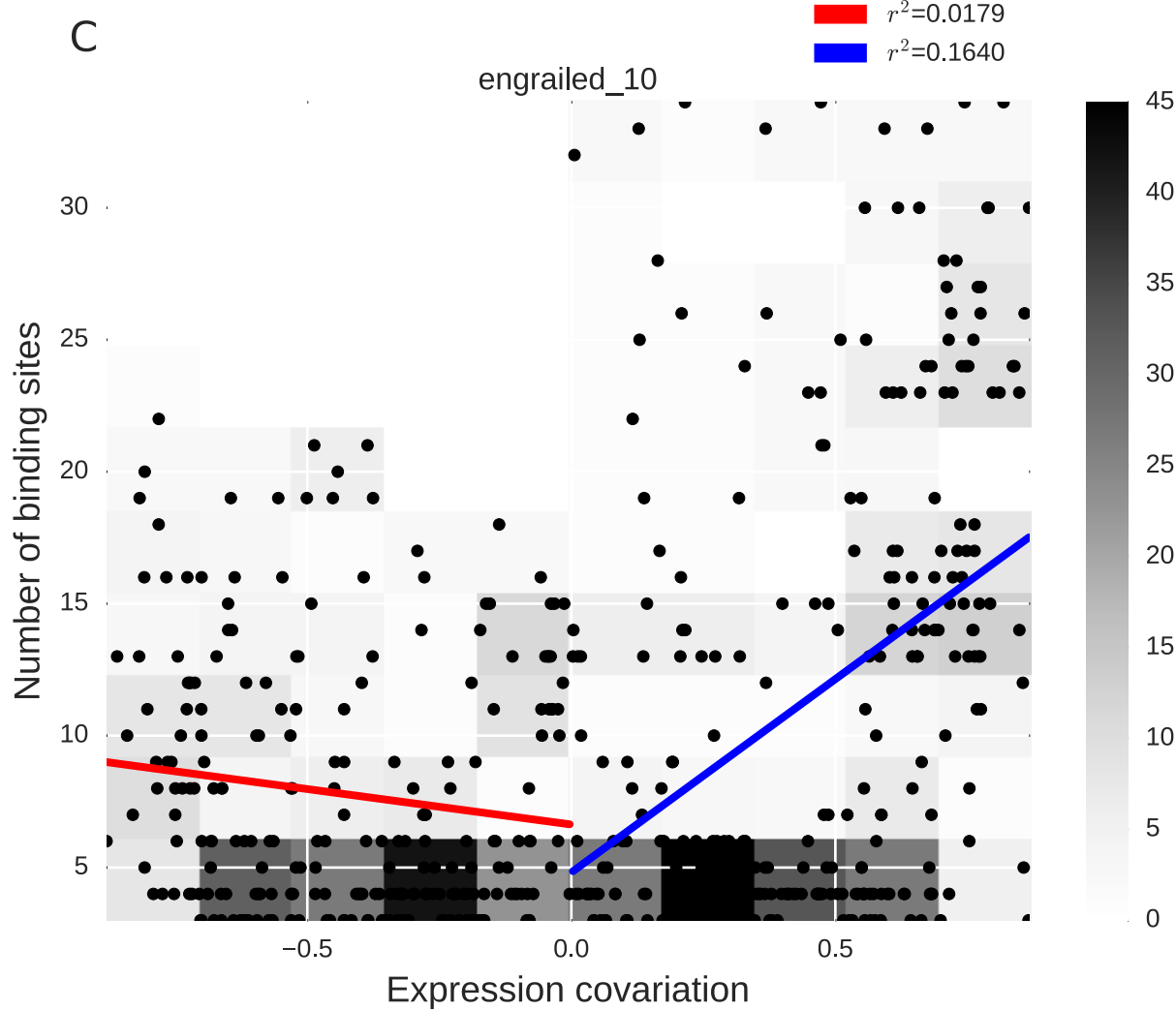
S3

A

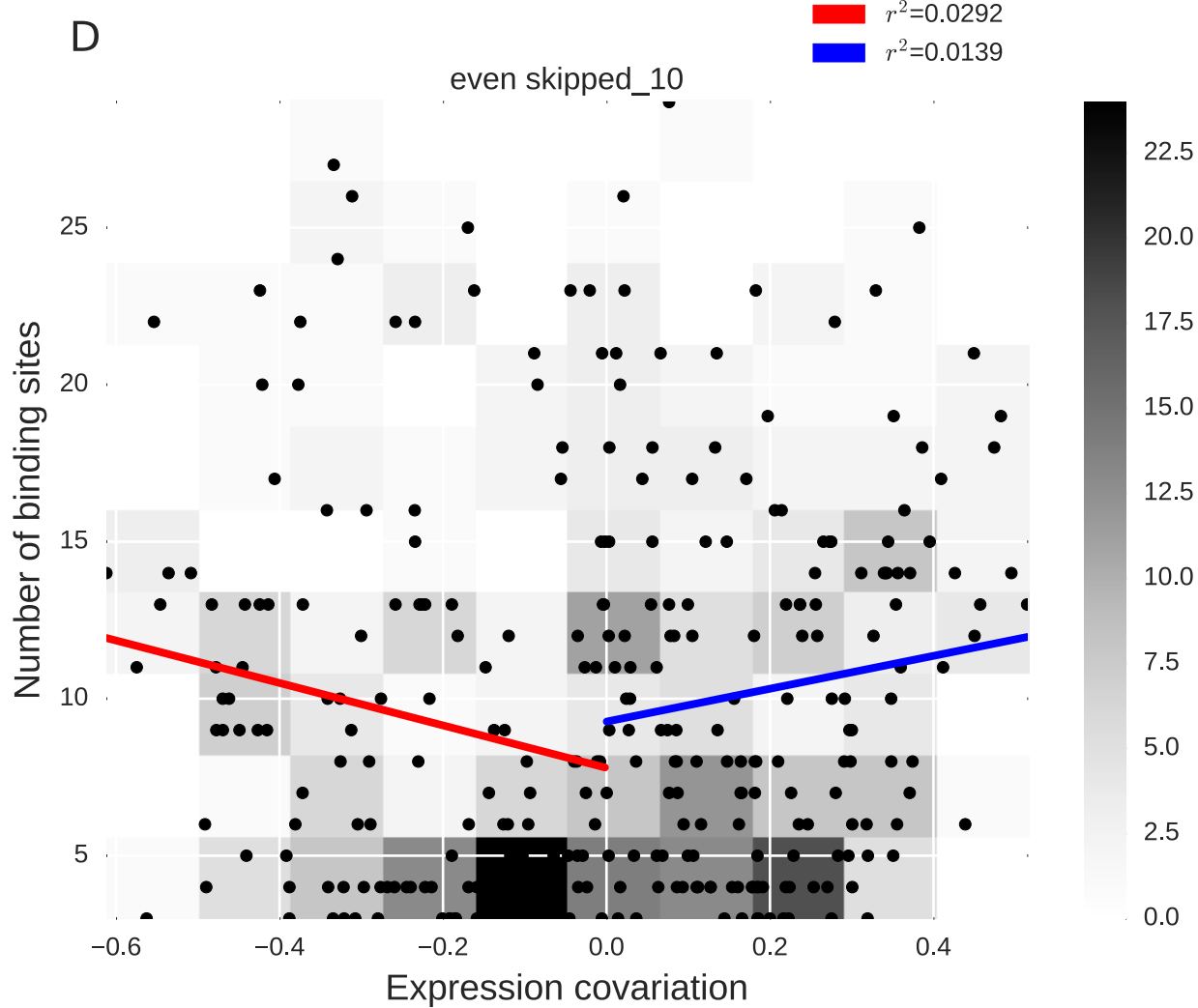


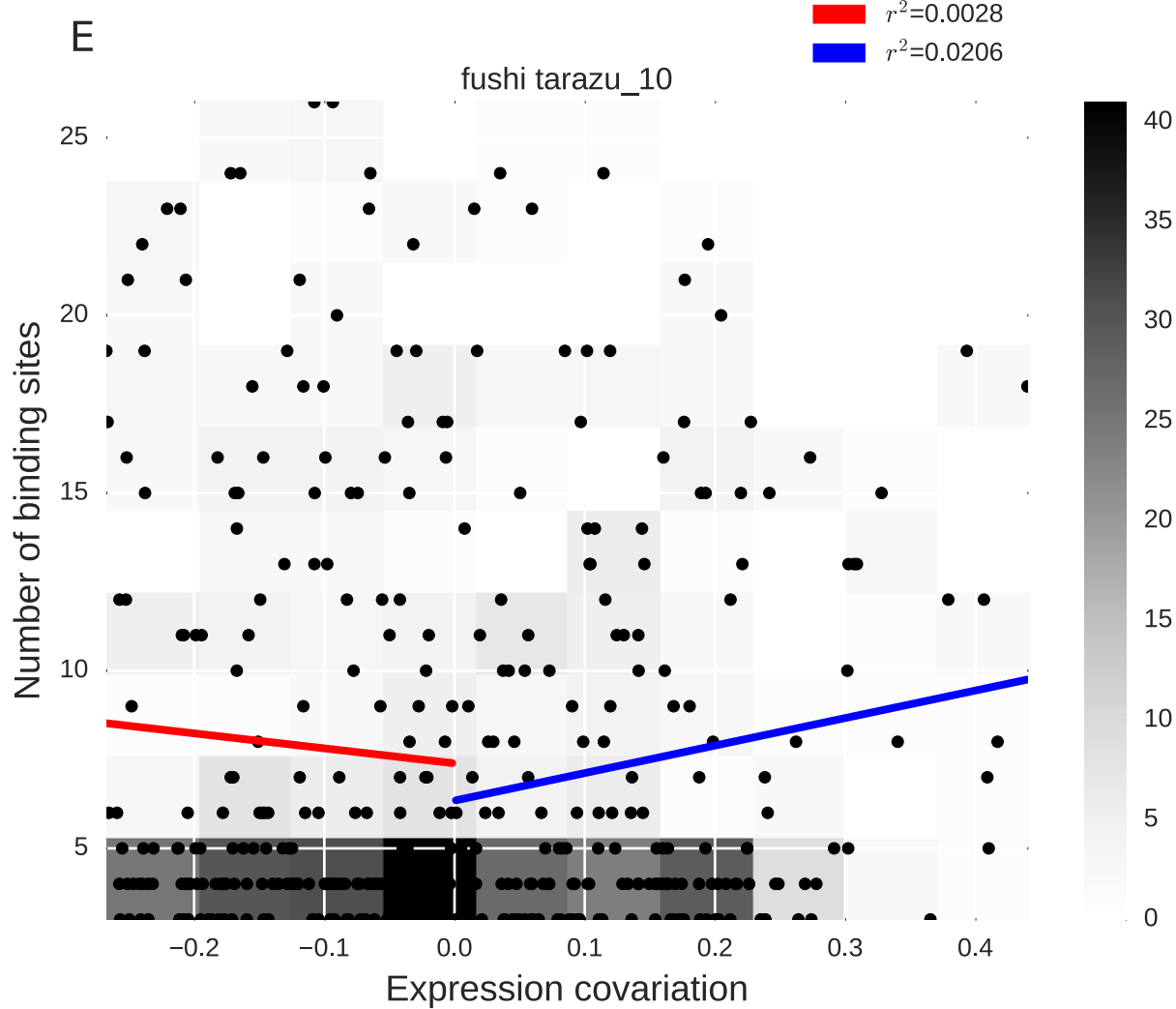


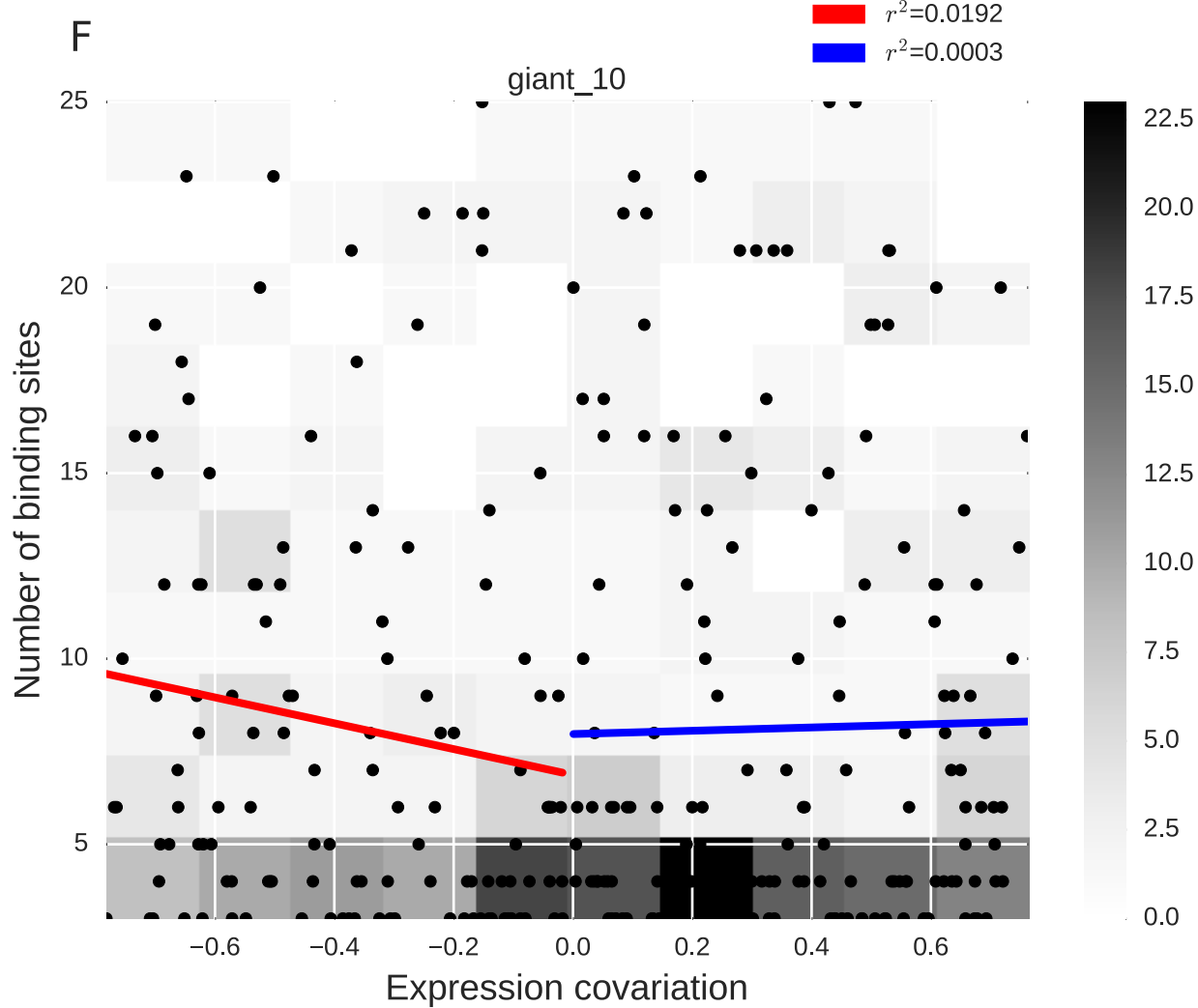
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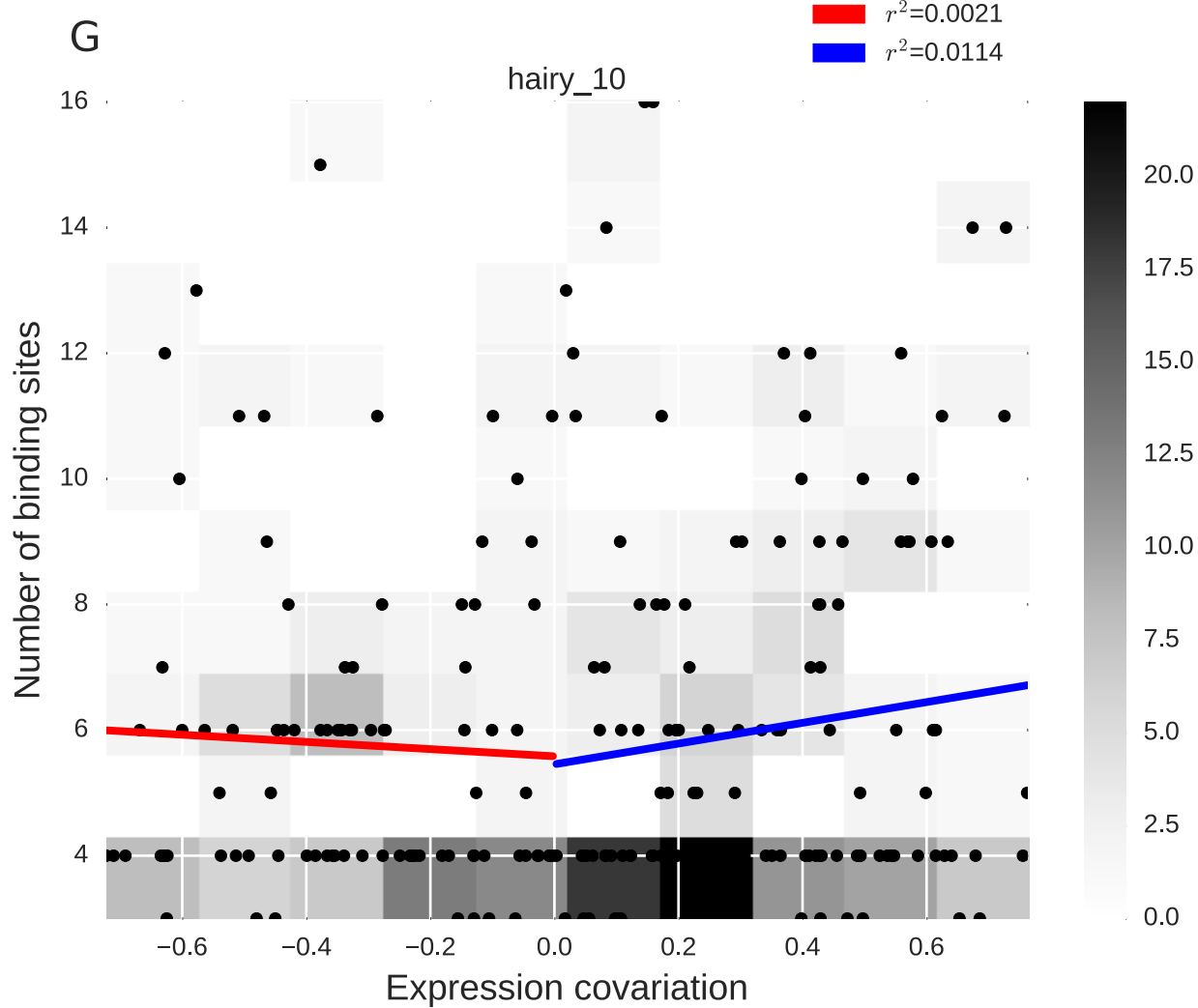


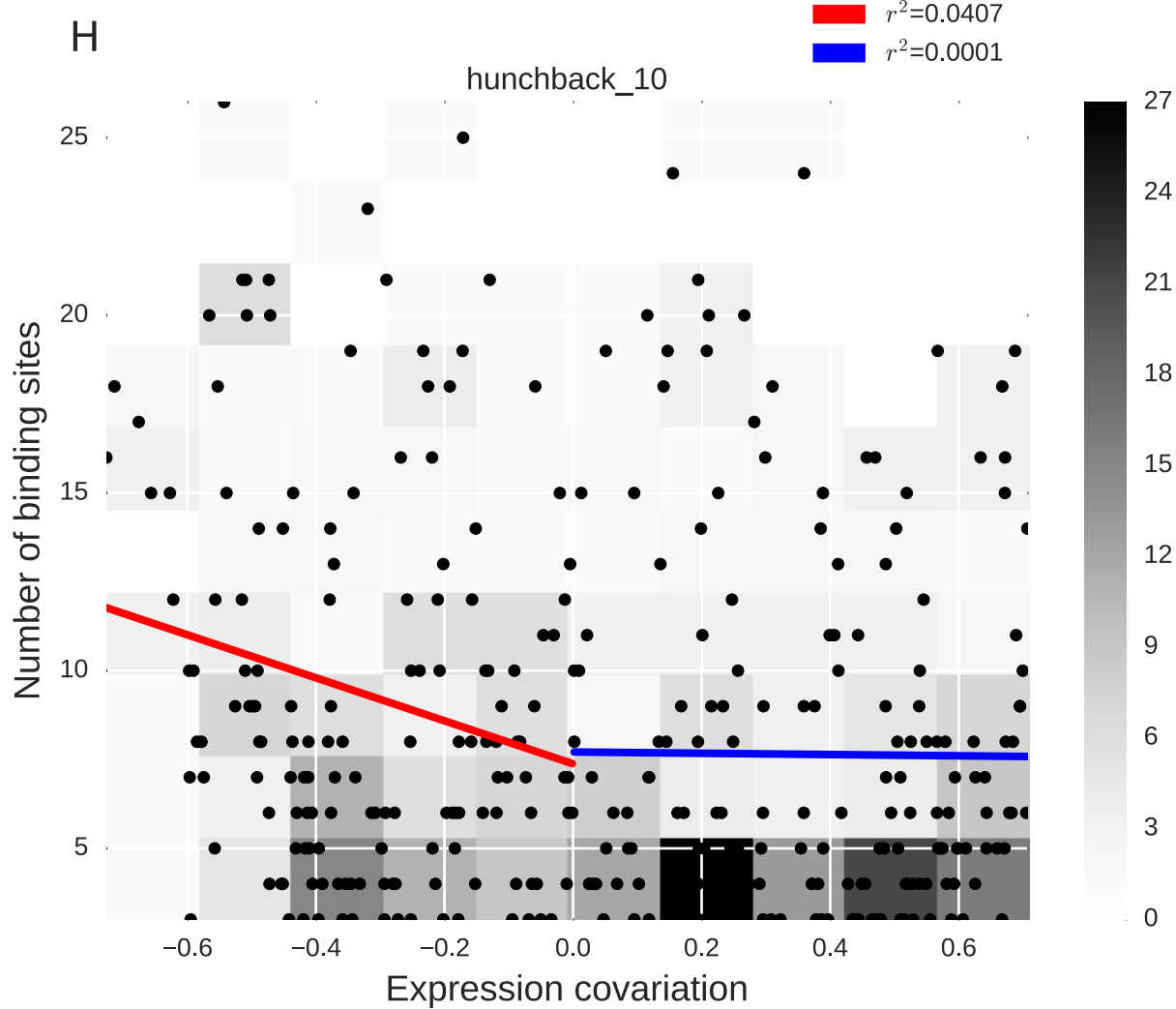
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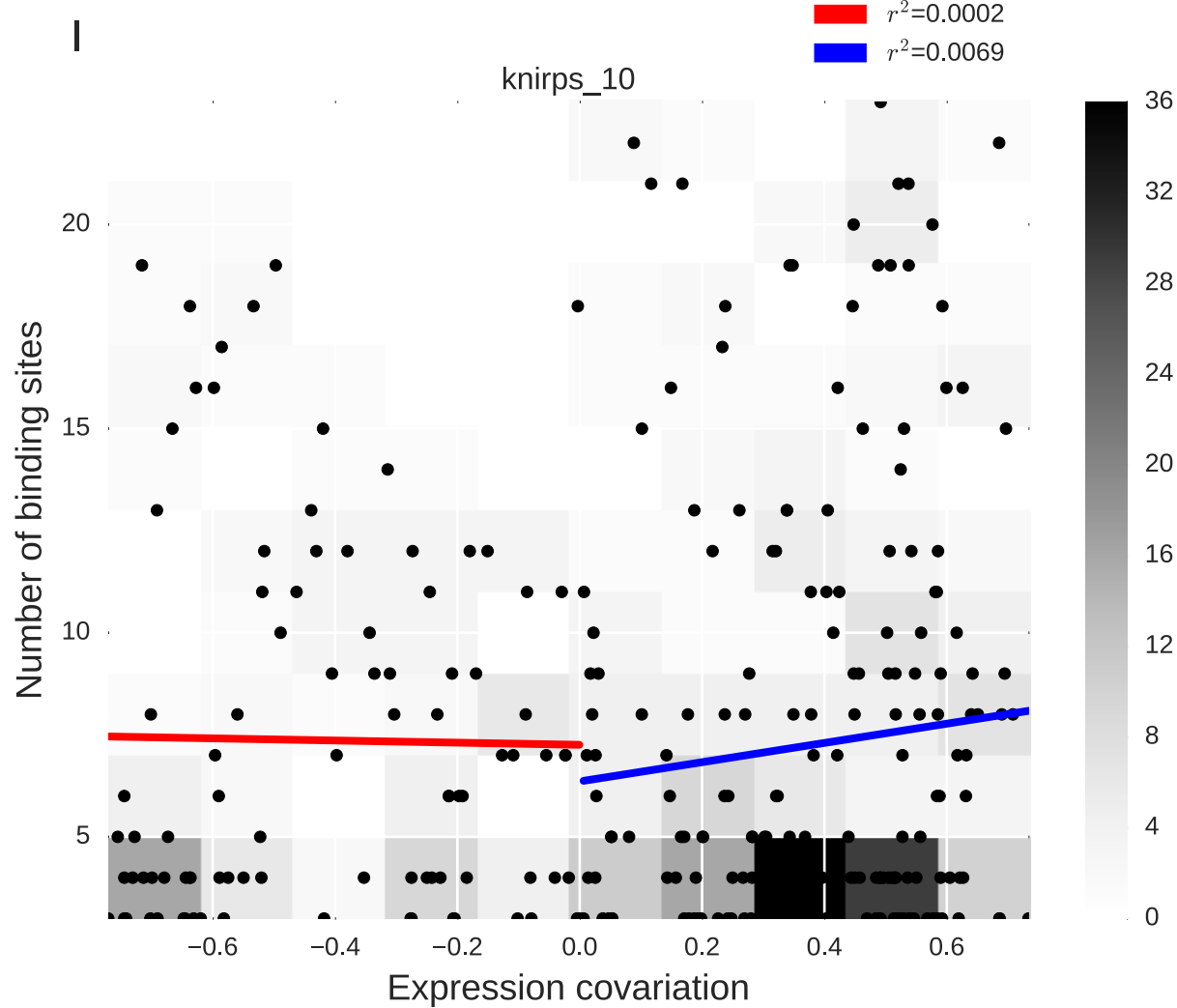


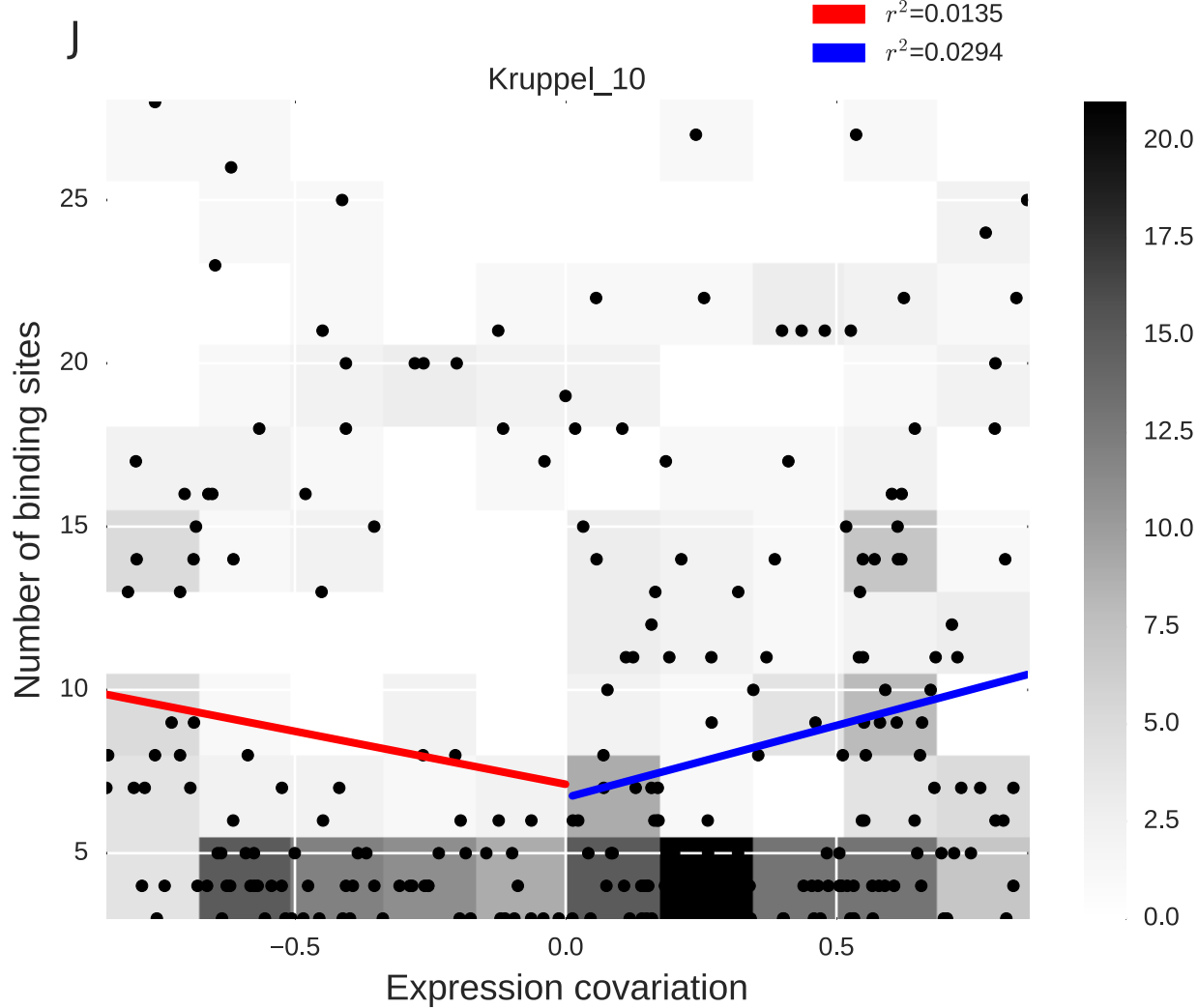


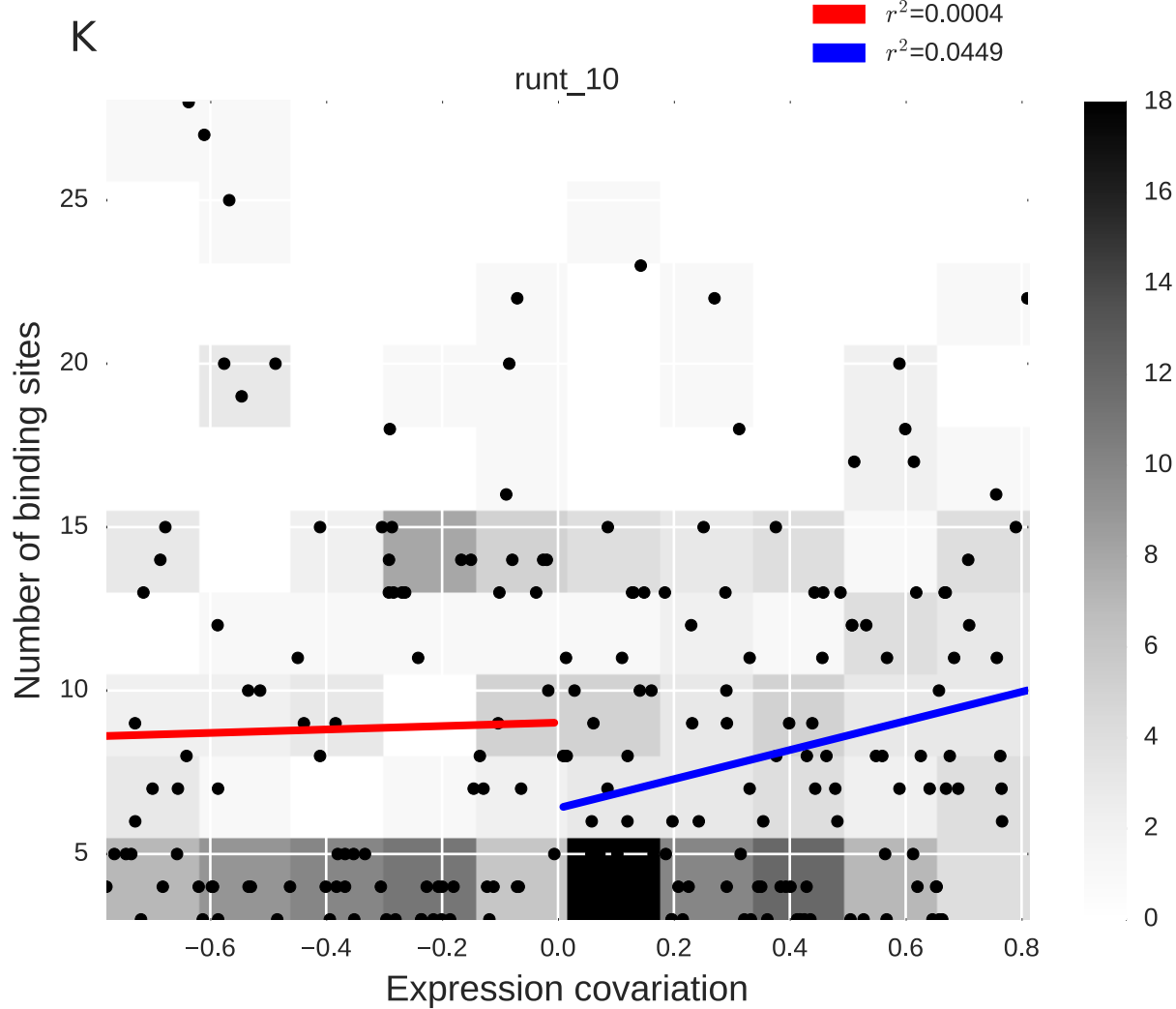


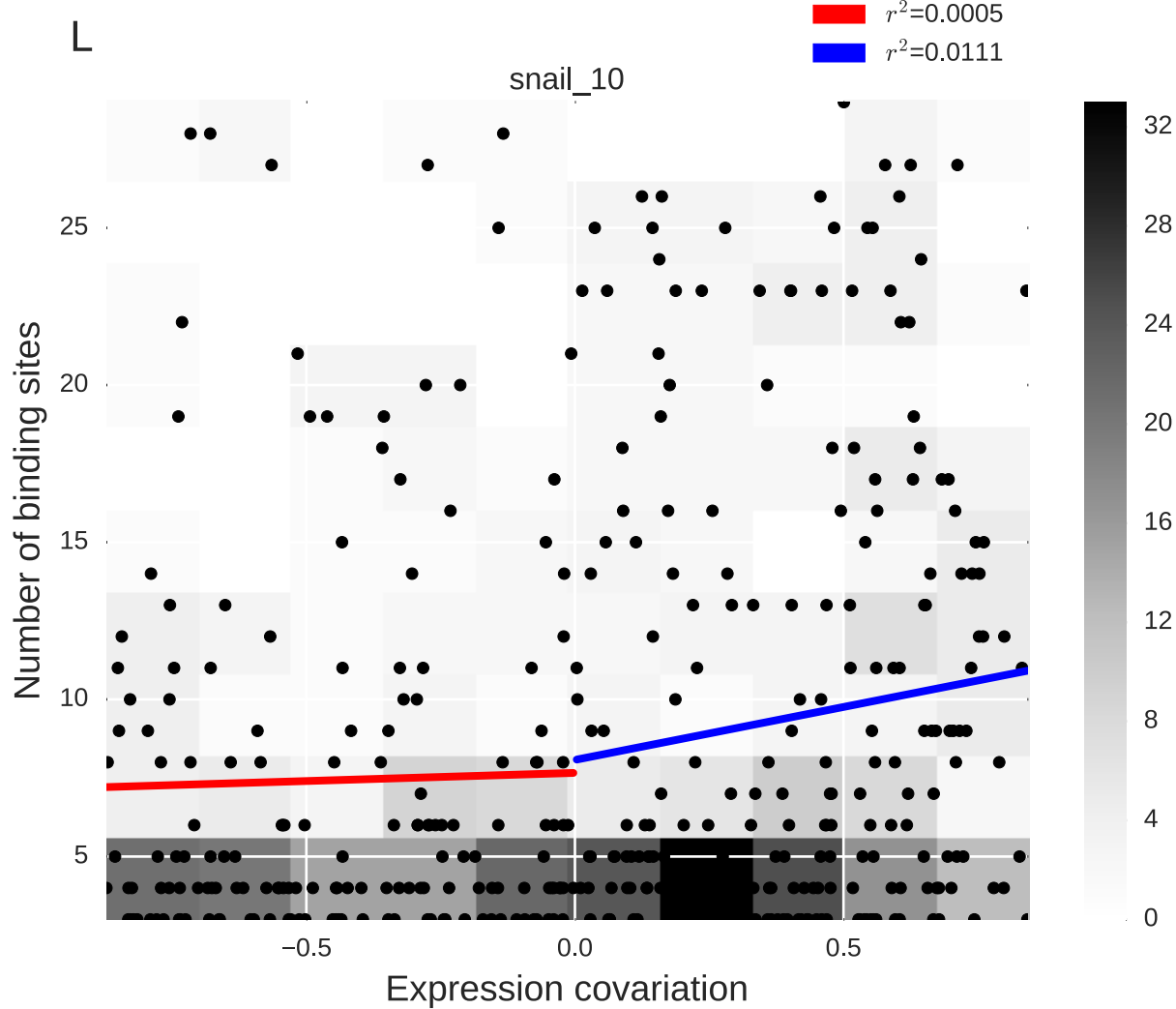


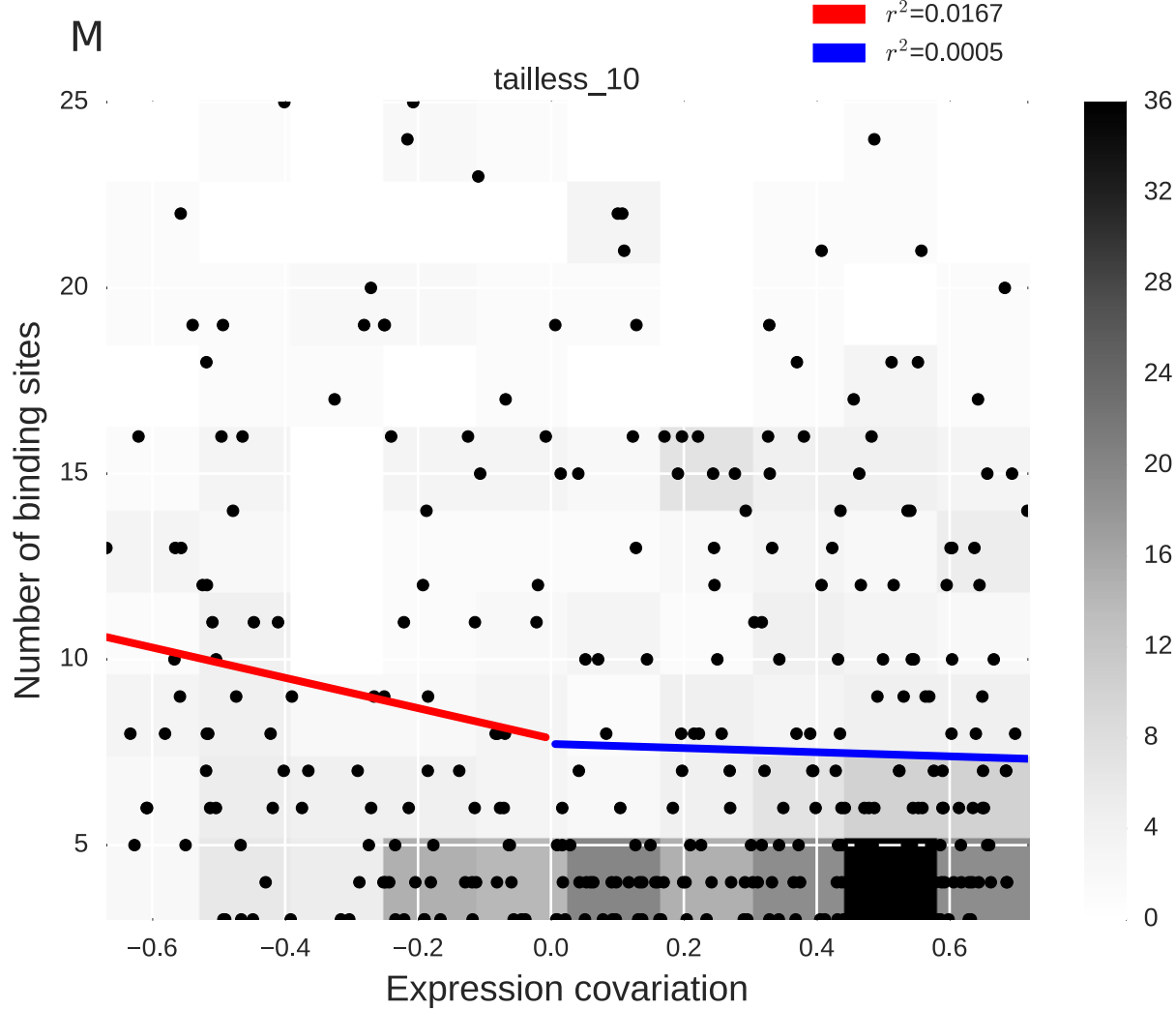


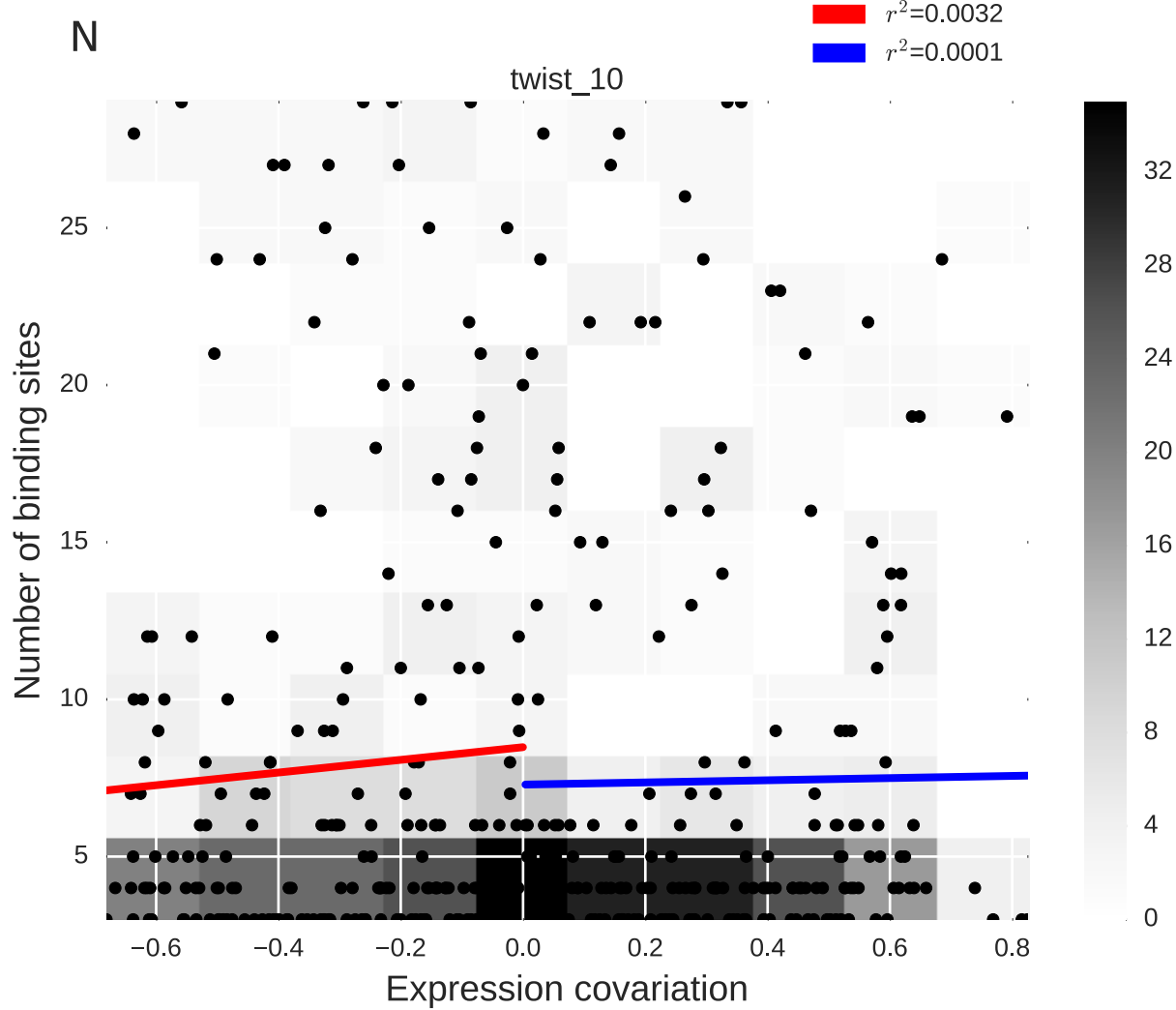




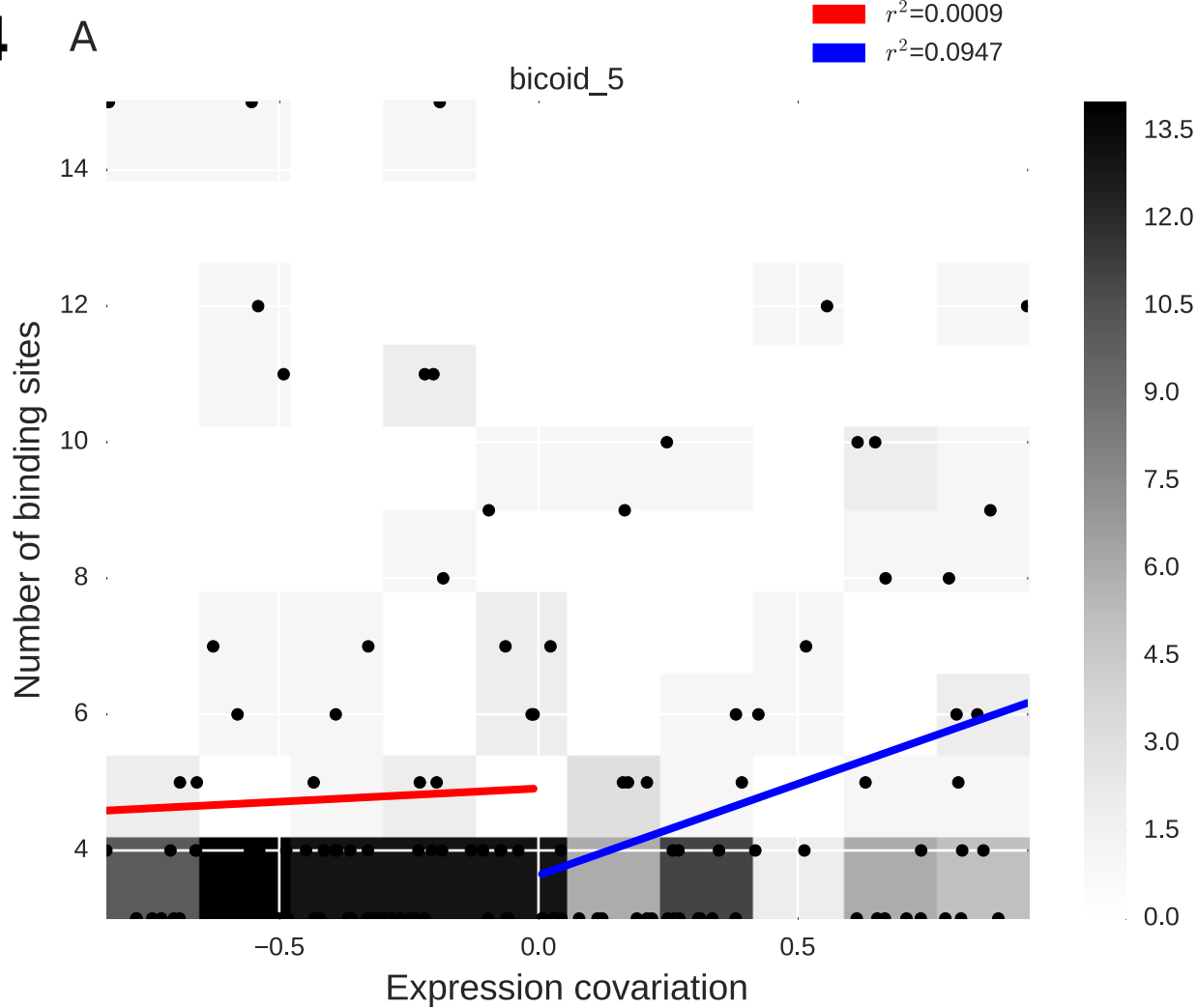


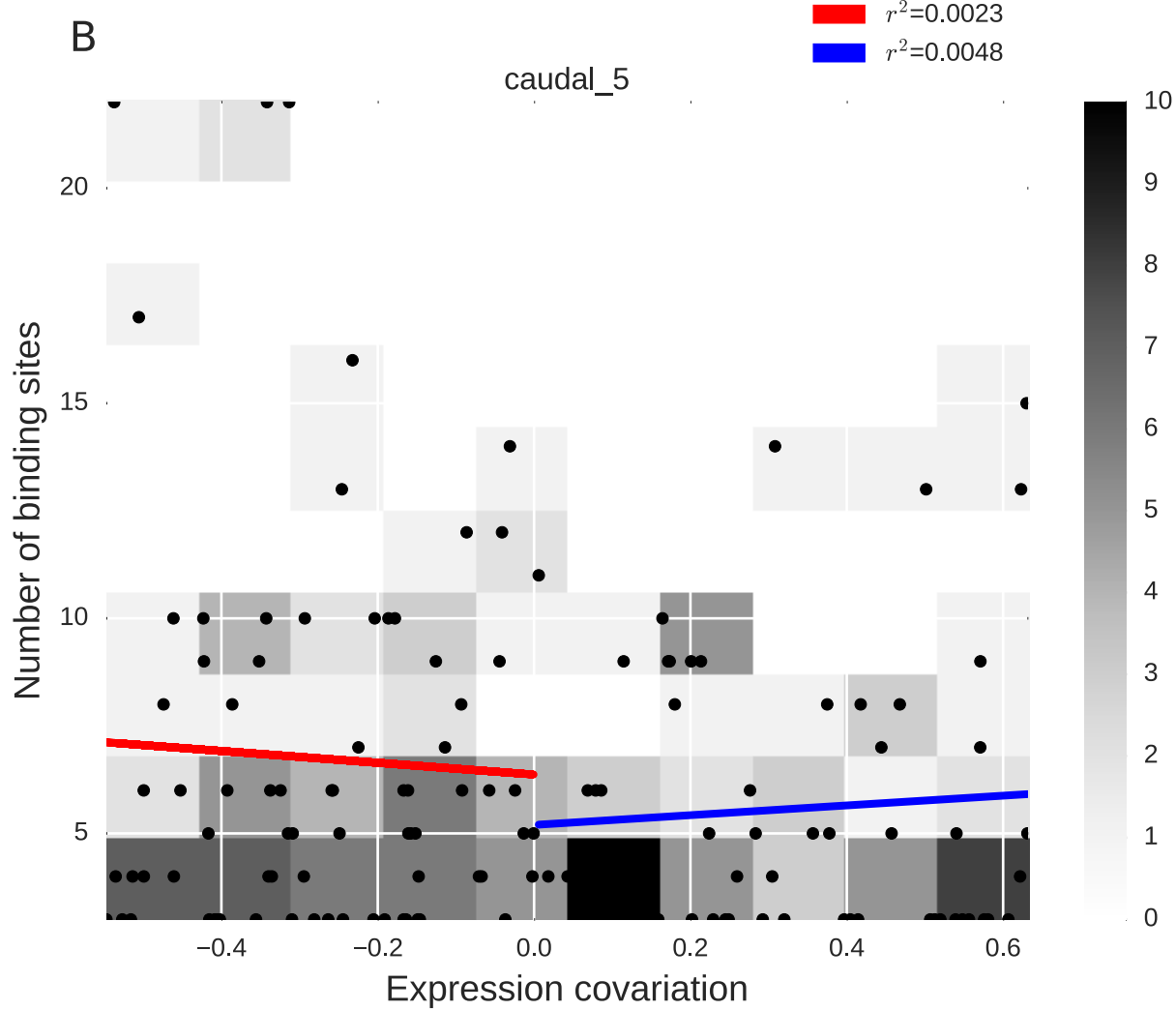




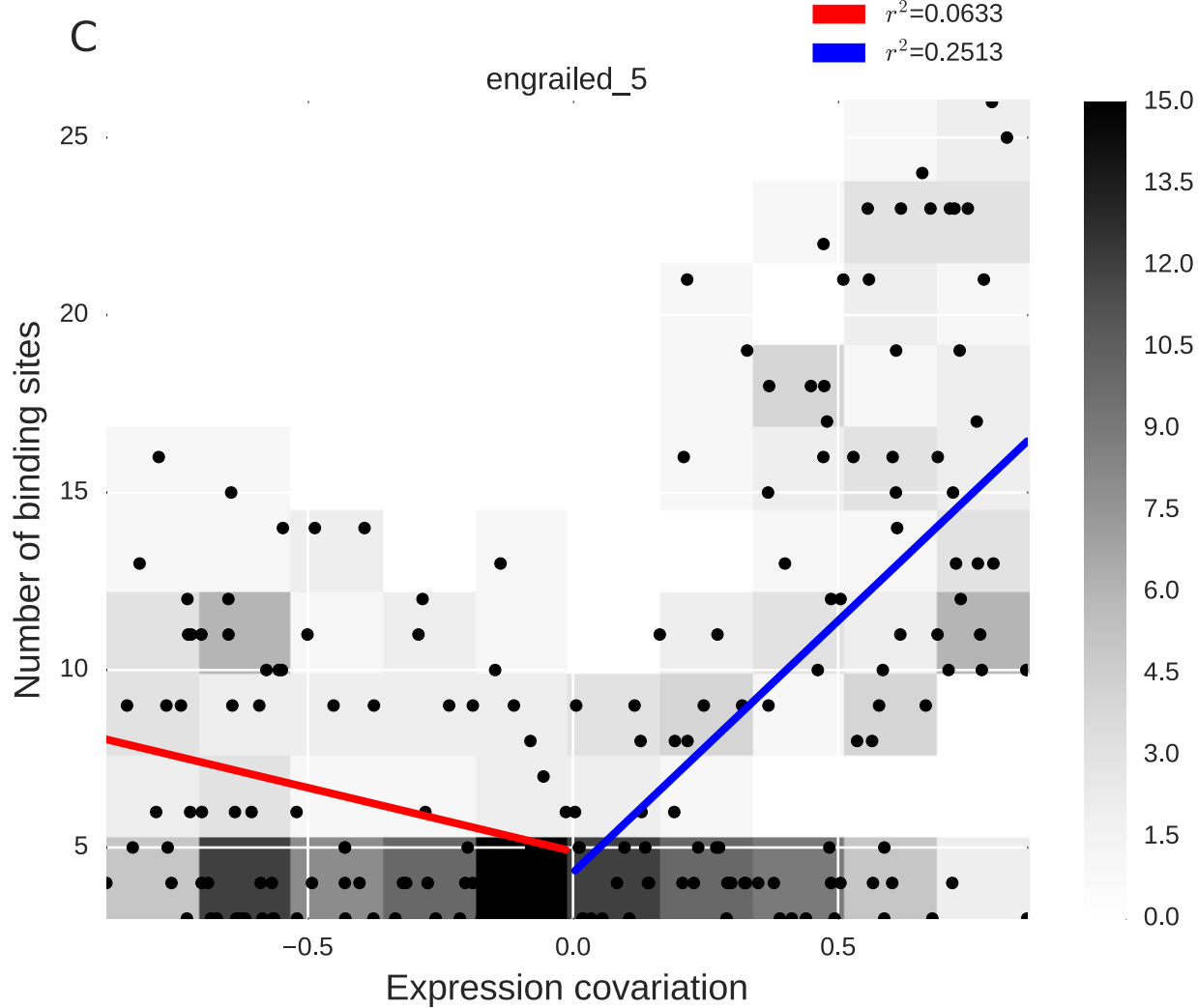


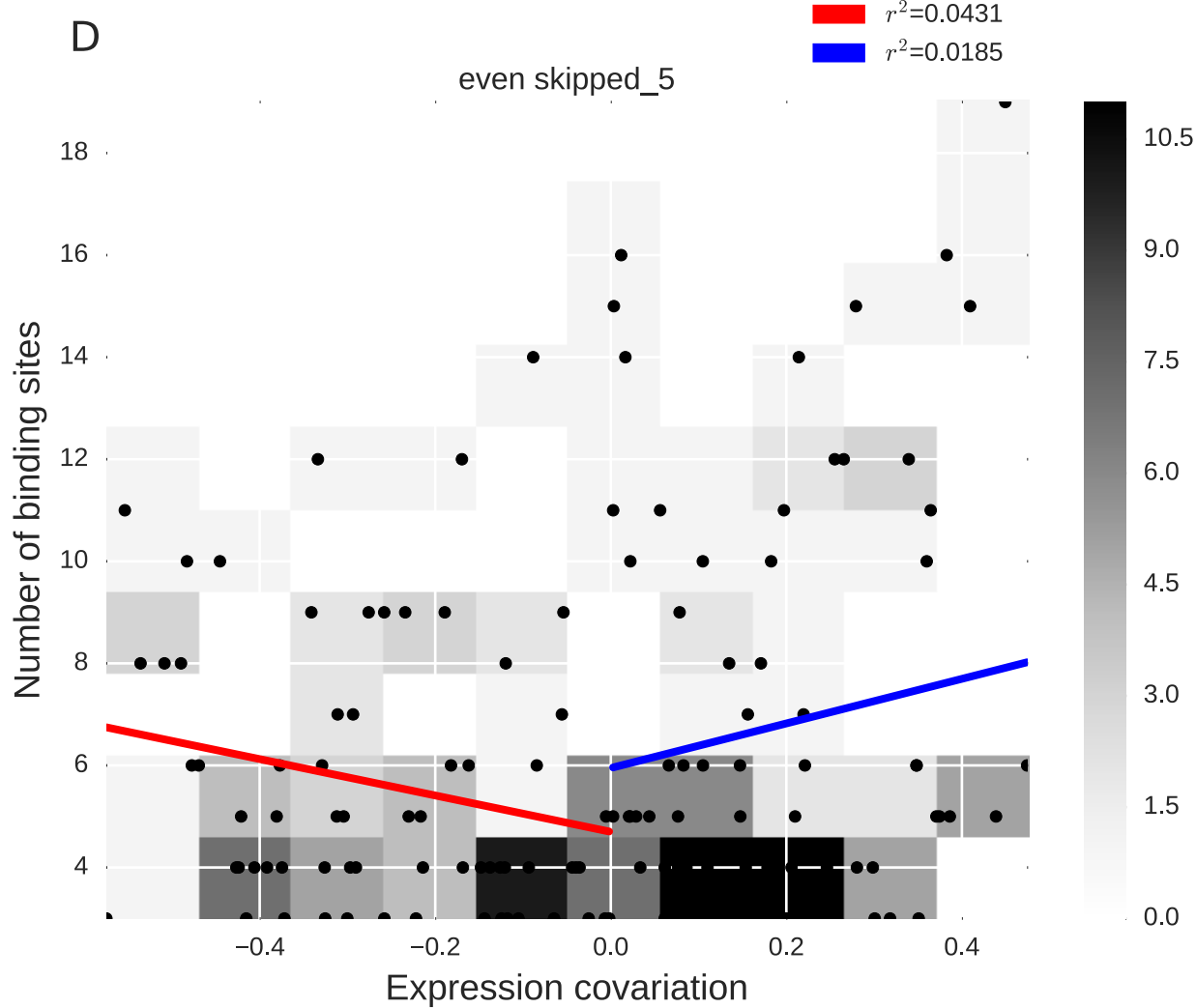
**Figure S4:** The relationship between regulatory strength and number of TF binding sites for target genes using the top 5% of binding scores for each TF. Here we plot the relationship between regulatory strength and number of TF binding sites for all our TFs of interest. Each black dot represents a target for the TF in the panel with at least three binding motifs. The x-axis shows the correlations between the expression level of each TF and its target genes across all our samples. The y-axis shows the number of TF binding sites which located in the assumed region of regulatory DNA for each target gene. The blue and red lines show the linear regression for the positively and negatively correlated target genes, respectively, and  $r^2$  values are displayed for these best fit lines.

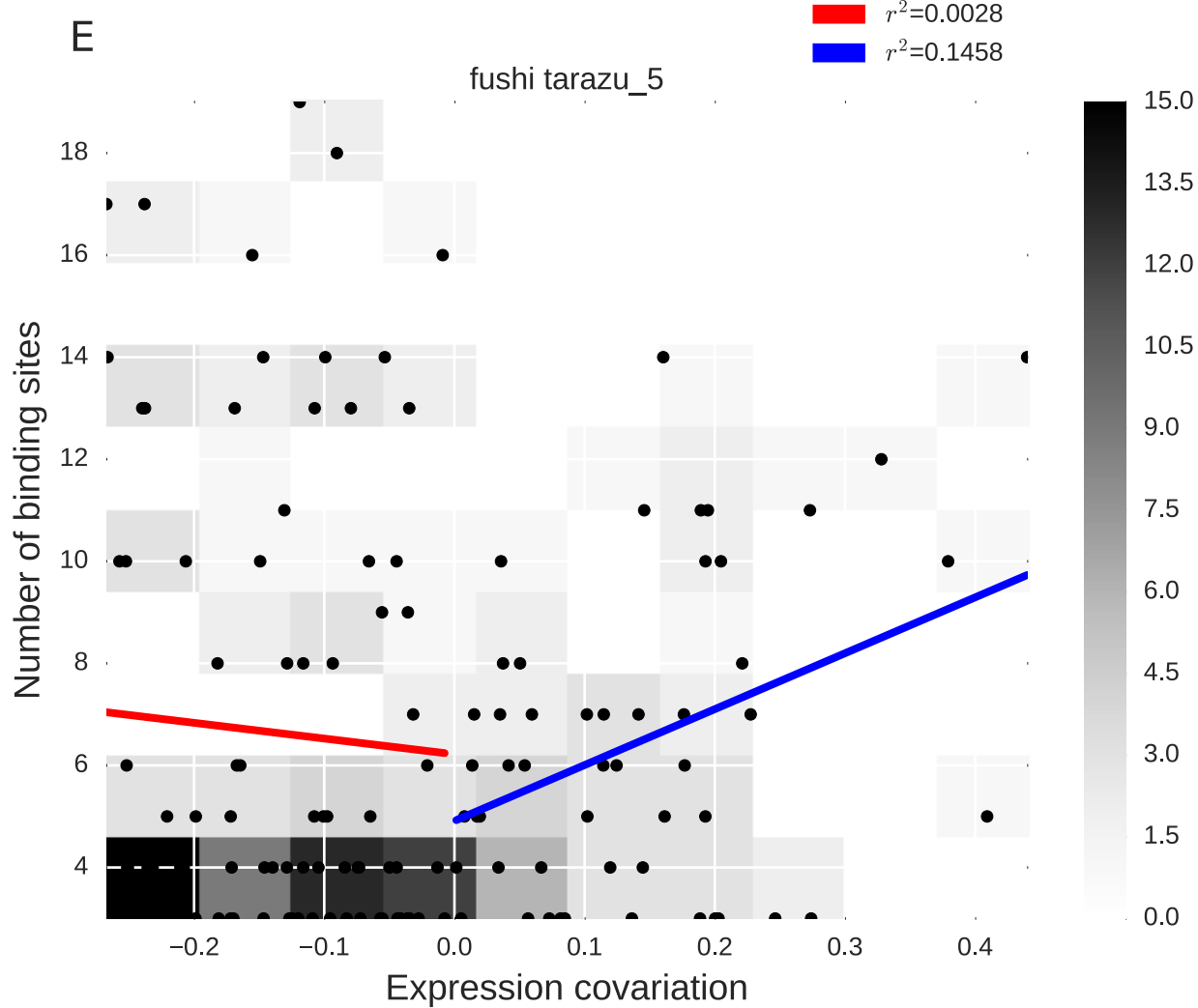
**S4****A**

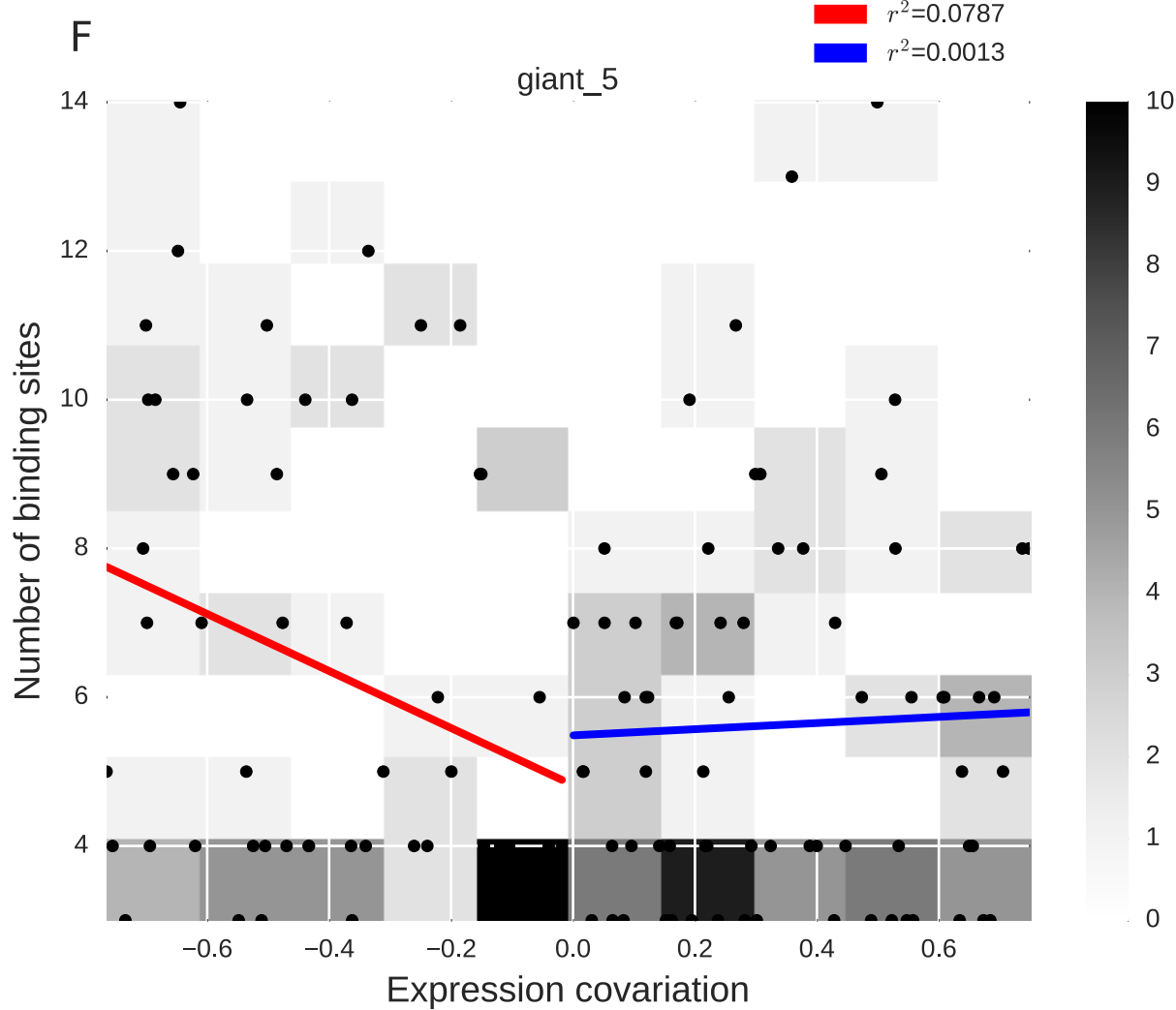


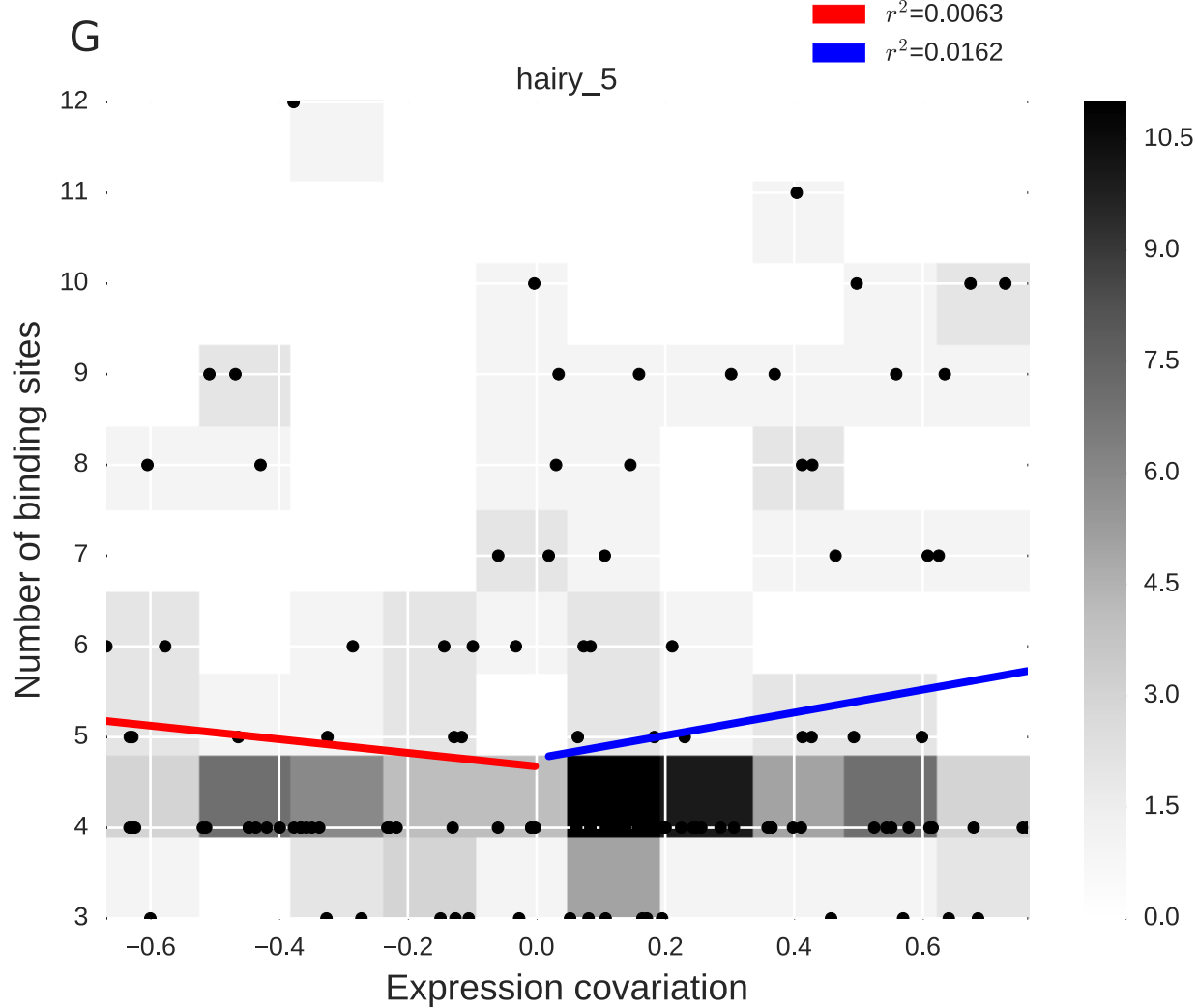
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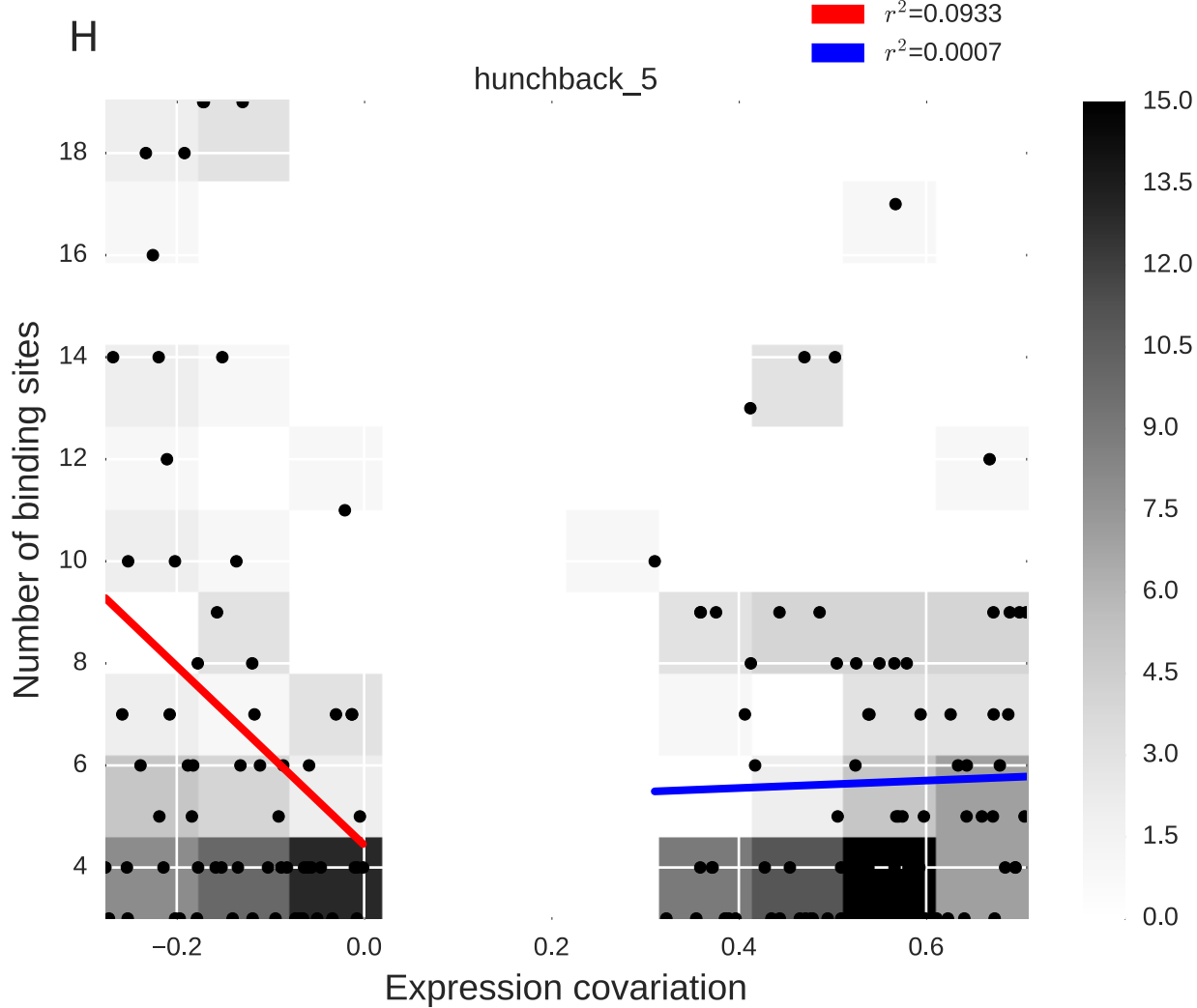


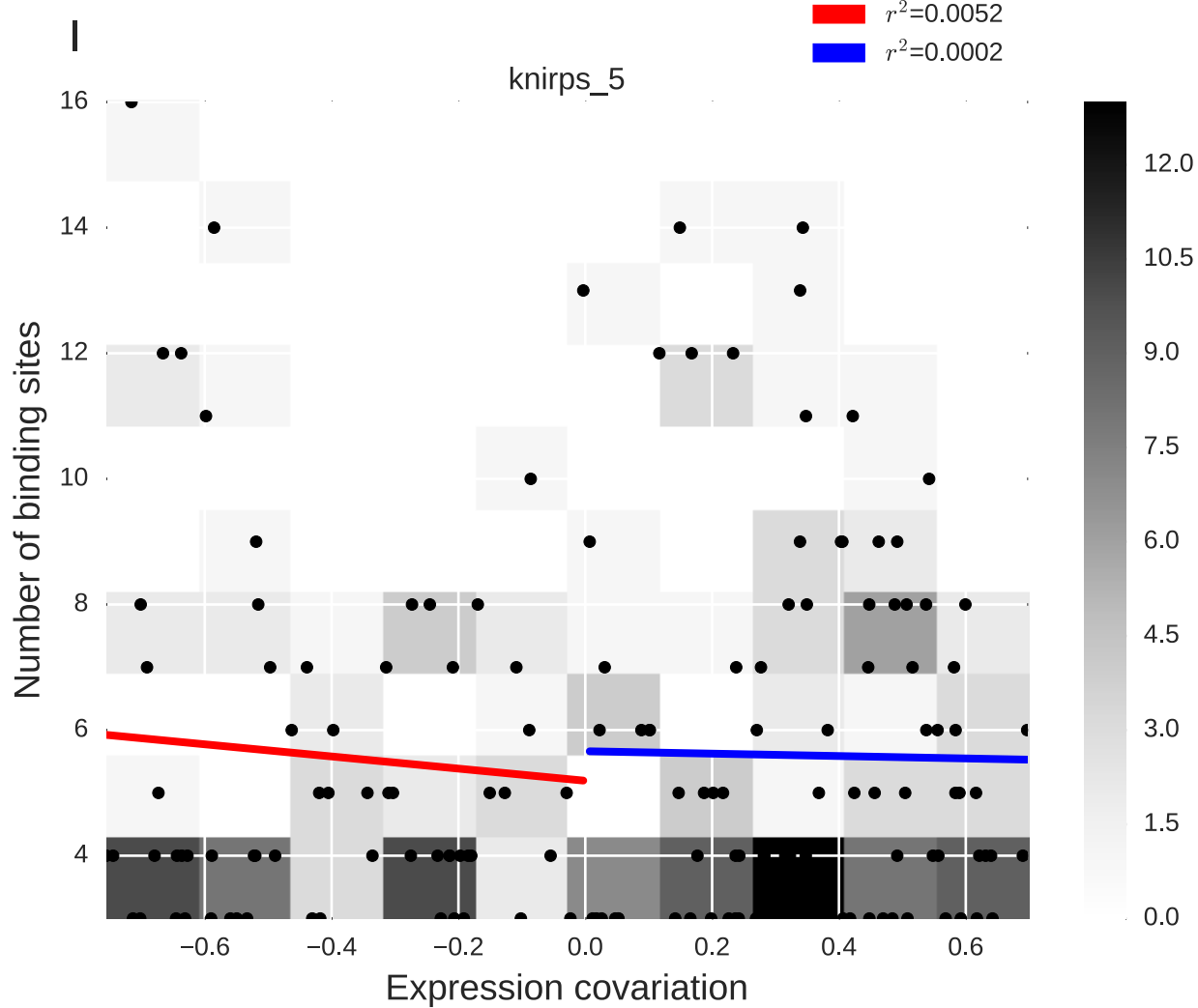


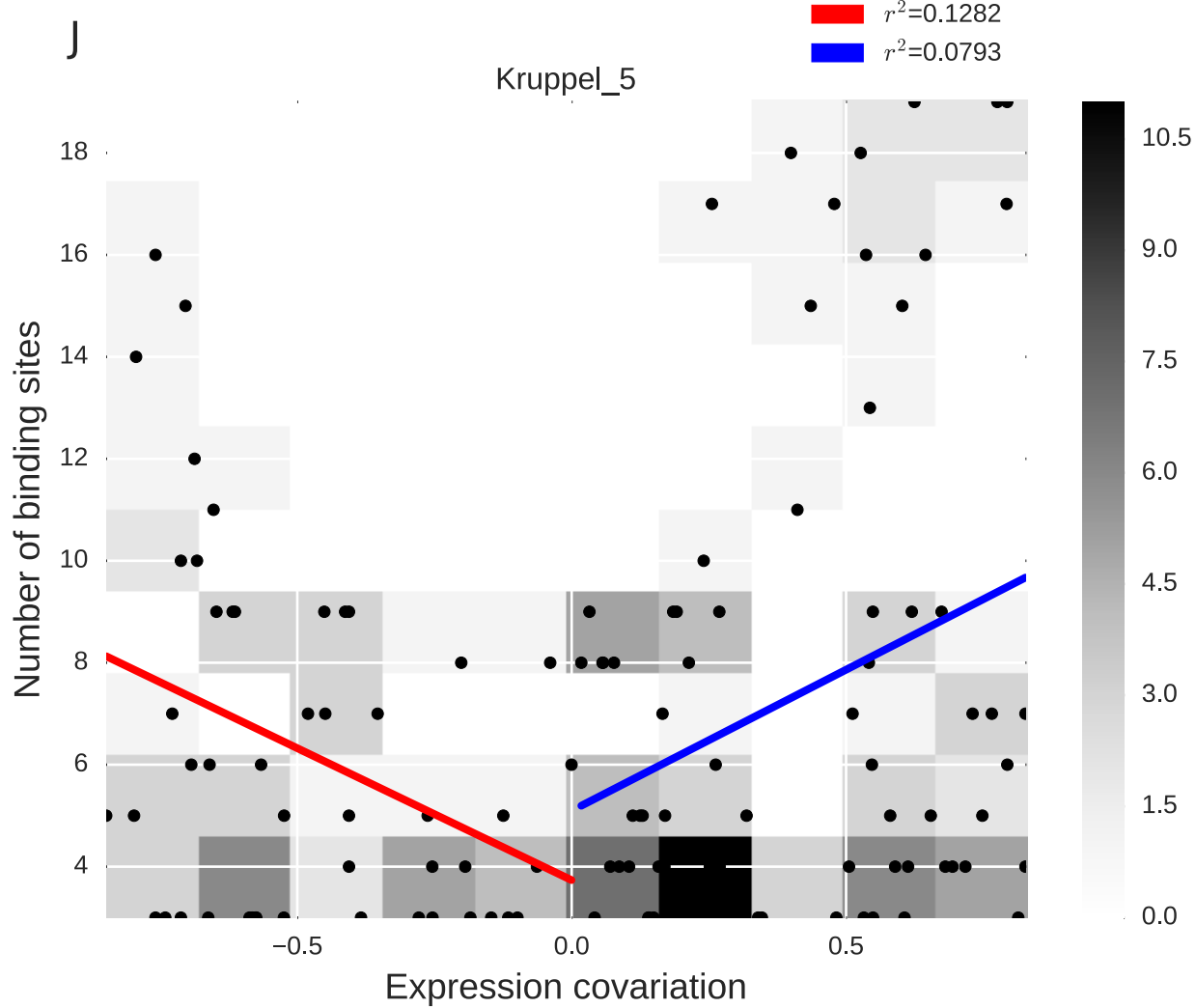


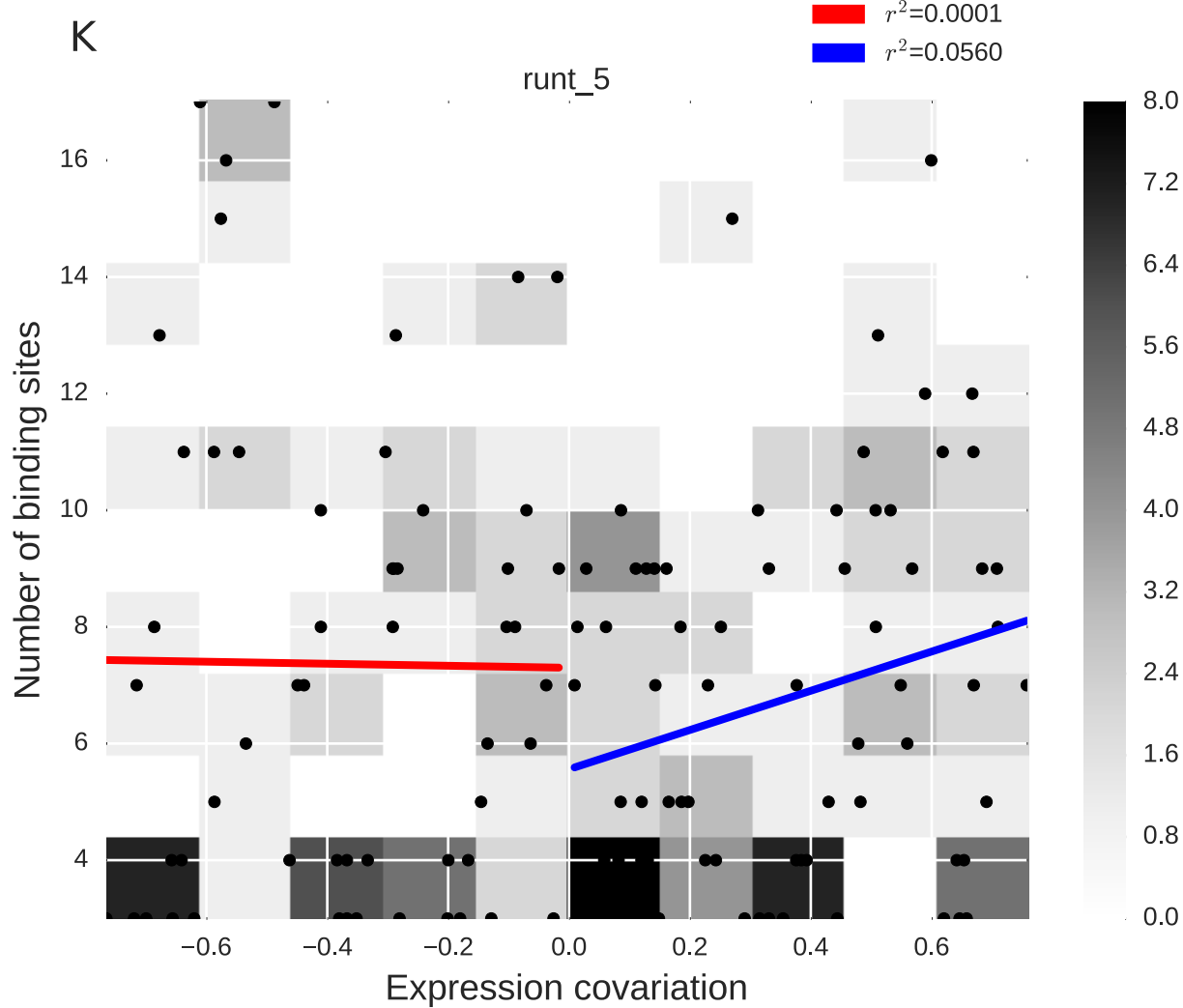


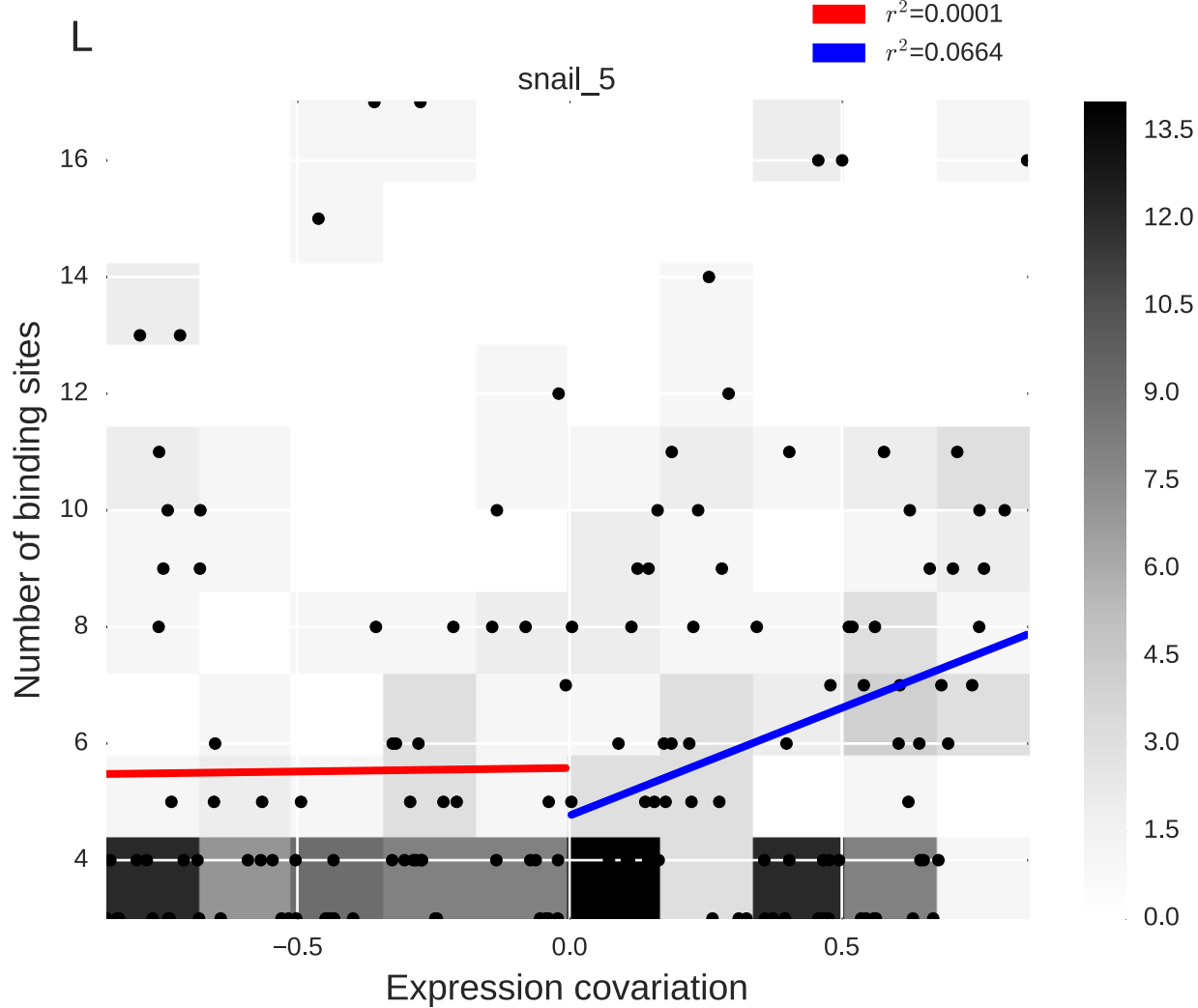


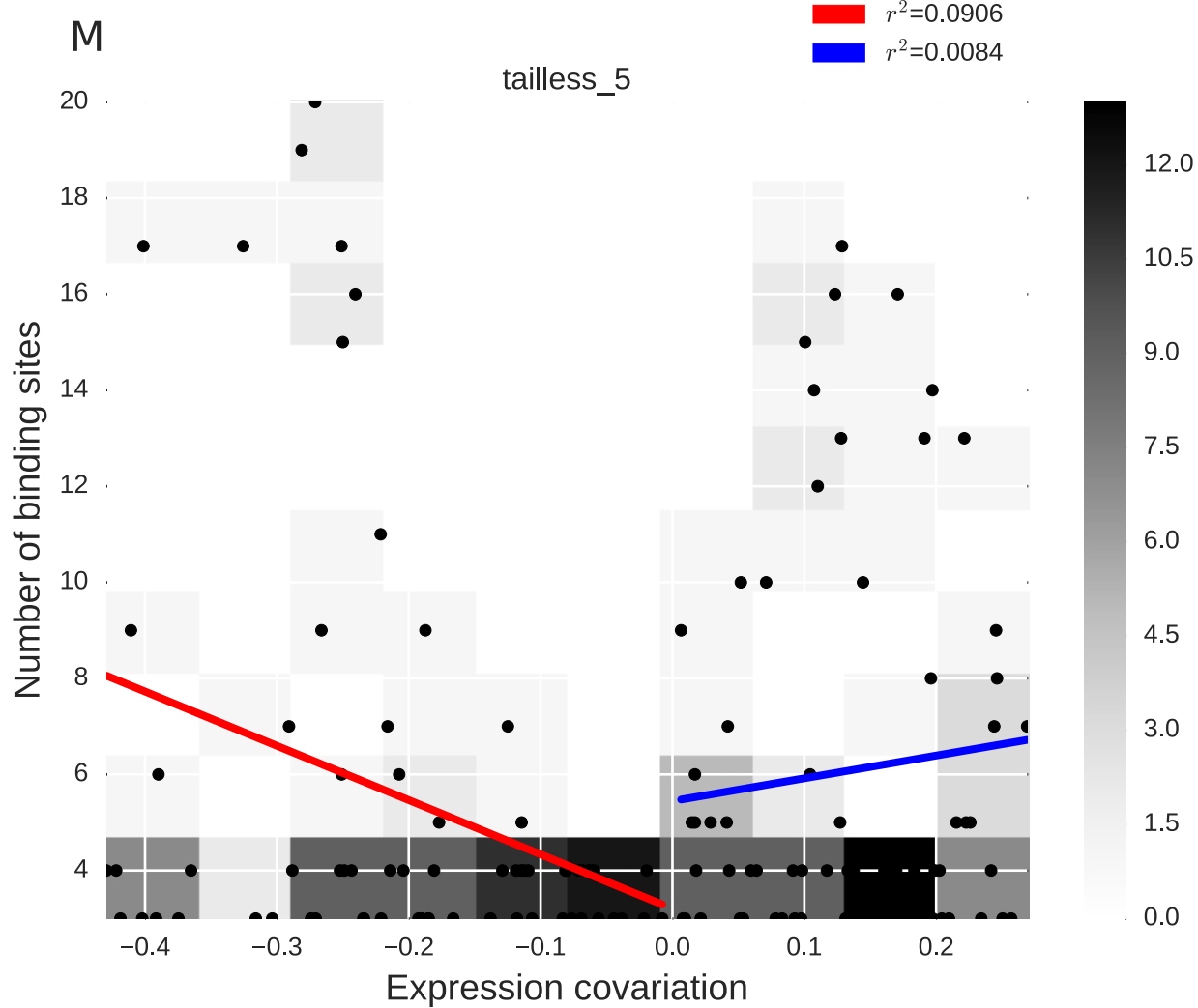


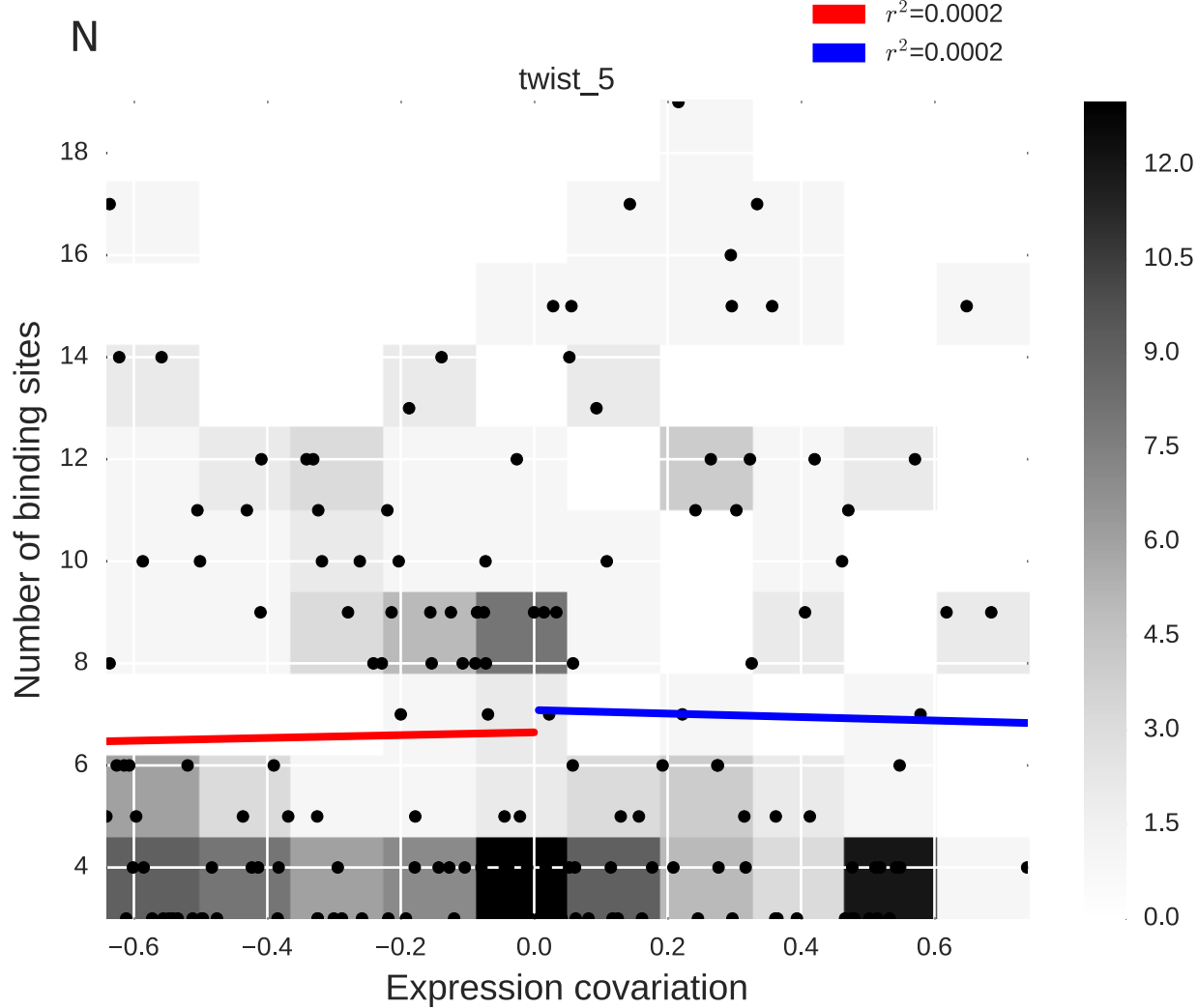




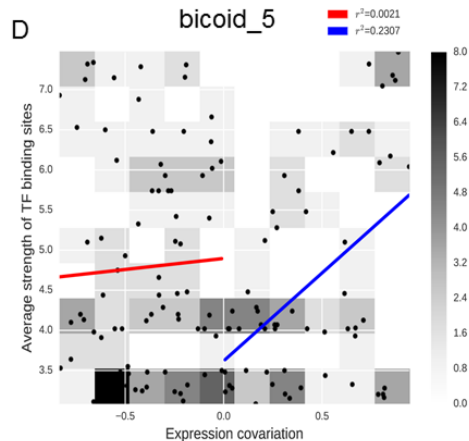
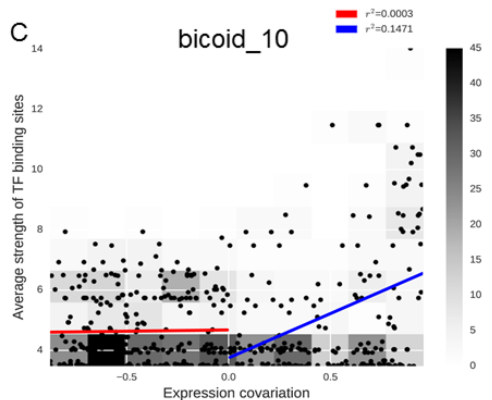
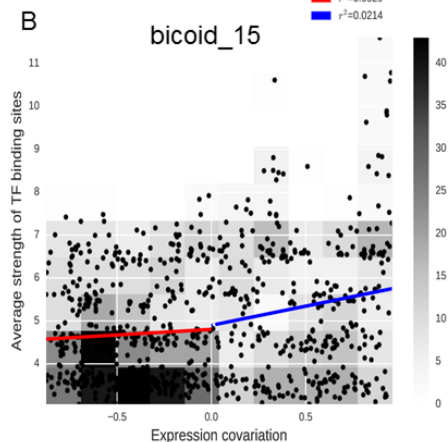
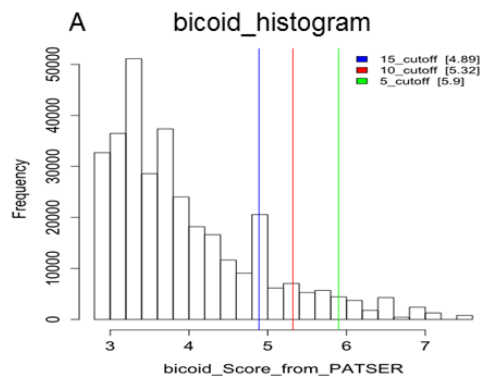






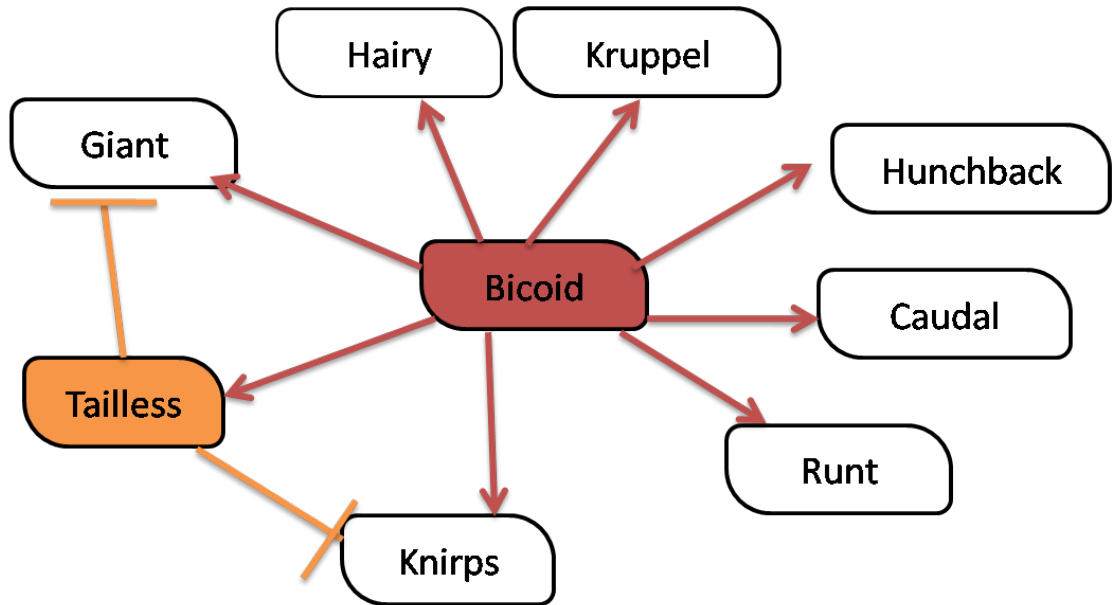


**Figure S5:** The relationship between regulatory strength and average bicoid's binding site strength for target genes using the top 15%, 10% and 5 % of bicoid's binding scores. (A) Here we plot the score cutoff values corresponding to the top 15%, 10% and 5 % of bicoid's binding site scores. (B, C, D) Here we plot the relationship between regulatory strength and average bicoid binding strength. Each black dot represents a target for bicoid in the panel with at least three binding motifs that fell into the top 15% (B), 10% (C), and 5 % (D) of binding site scores. The x-axis shows the correlations between the expression level of bicoid and its target genes across all our samples. The y-axis shows the average binding site strength for bicoid's binding sites located in the assumed region of regulatory DNA for each target gene. The blue and red lines show the linear regression for the positively and negatively correlated target genes, respectively, and  $r^2$  values are displayed for these best fit lines.



**Figure S6:** Regulatory interaction between TFs. Red arrows represent positive correlations between *bicoid* and other TFs. Orange T-bars represent negative correlations between *tailless* and two gap genes (*giant* and *knirps*) and green T-bars represent negative correlations between *twist* and *odd paired* and *sloppy paired*.

# S6



**Figure S7:** Results are robust to the genotype of the tester strain. Crosses using two tester lines (R380 and R315) show similar correlation coefficients for the TFs tested. The x- and y-axes are columns 3 and 4 of Table S5, respectively.

# S7

